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THE DEVELOPMENT OF WAIKATO PEAT SOILS

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Summary

The characteristics of peat which have contributed to difficulties in its development in the Waikato district are discussed.

Analytical data and results of observational field topdressing experiments are given. These show that:—

1. The peat has a high C/N ratio and is deficient in available nitrogen. Nitrogenous fertilizers gave substantial but short-lived increases in the growth of grasses but depressed clovers. The use of nitrogenous fertilizers alone is not the key to the successful development of the peat.

2. Growth of white clover on unimproved acid peat topdressed with lime only was very poor but increased slightly with pH. With serpentine superphosphate and muriate of potash, growth was good at pH 4.5 but decreased when the pH was raised to 5.0 or higher. Growth was negligible below pH 4.0, with or without added phosphate or potash.

3. On semi-improved peat (pH 5.1), mixtures of 10 cwt per acre of serpentine superphosphate and 5 cwt per acre muriate of potash gave excellent responses on mixed pasture, chiefly in the white clover. Phosphate and potash when applied separately at these rates gave negligible responses. The minimum quantity of the mixture required to give good responses was 4-7 cwt serpentine superphosphate and 2-5 cwt muriate of potash.

The possibility of facilitating the development of peat land by promoting clover growth through heavy initial applications of phosphate and potash and thus increasing the available nitrogen sufficiently to grow the best species of pasture grasses, is discussed.

Note.—Since the paper was written, subsequent experiments have shown that very good pasture responses may be obtained with considerably smaller quantities of fertilizers than those quoted above.

INTRODUCTION

Peat soils are of frequent occurrence in many countries of the world but vary greatly in their physical and chemical attributes. They possess in common, however, the characteristic of containing large amounts of undecomposed organic matter.

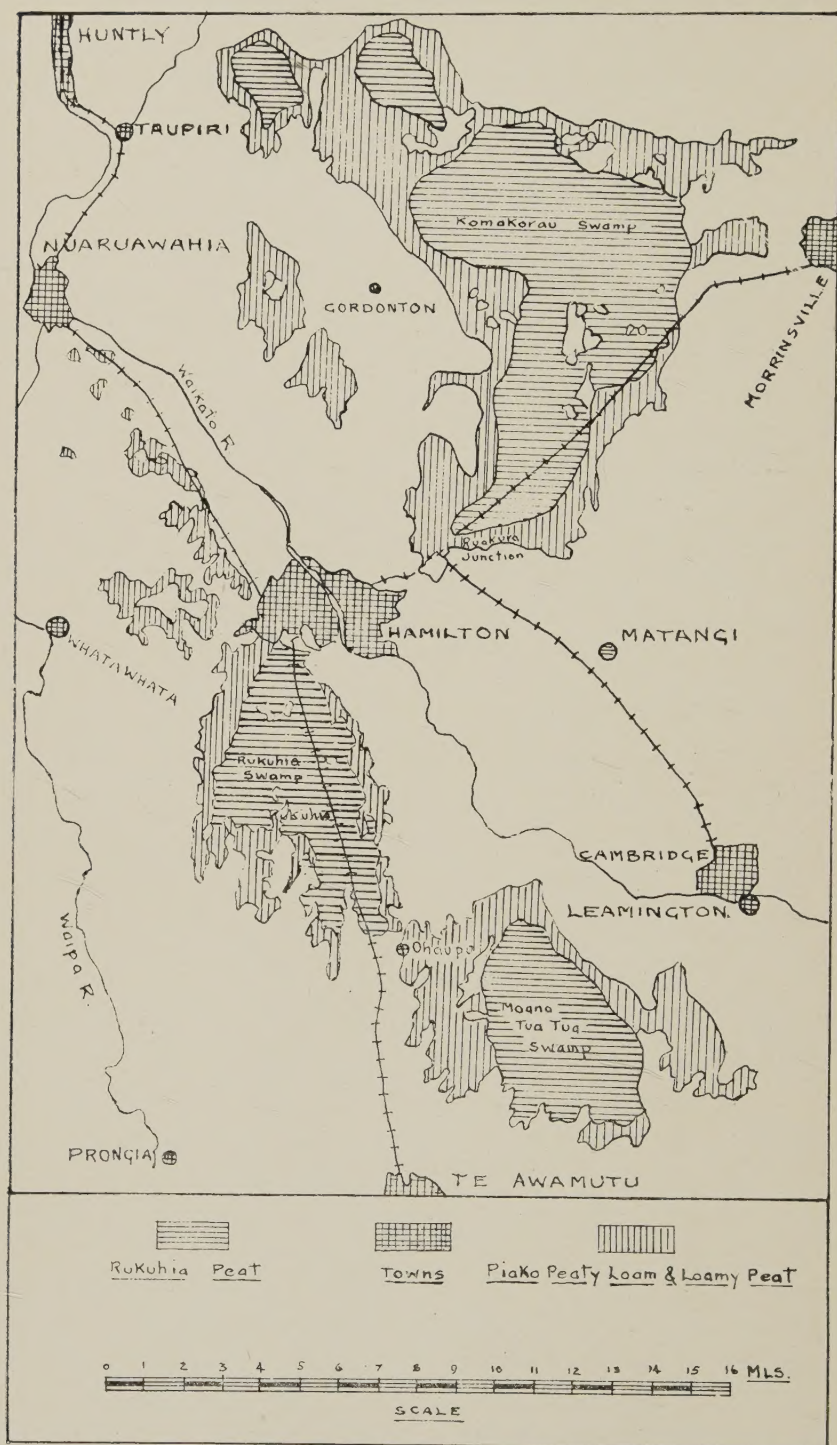


FIG. 1.—Map of the Waikato Basin showing the Moanatuatua, Rukuhia, and Komakorau Peat Swamps.

In New Zealand, peat soils occur in relatively small areas except in the Waikato basin where, according to figures supplied by the Soil Bureau, Department of Scientific and Industrial Research, they are estimated to cover an area of more than 100,000 acres, half of which is undeveloped. Peat also occurs in small isolated areas in most of the other provinces of the Dominion.

The importance of the peat soils in the Waikato basin lies largely in their geographical position (see Fig. 1). They are in the centre of a rich dairying district and lie within easy reach of up-to-date transport facilities, electric power, and most of the amenities which characterize present day farming in areas close to cities and towns. The draining of the peat swamps would make possible the settlement of large areas of land at present unproductive or only partly productive, and may appreciably affect the climate of the Waikato basin.

In the past, a great deal of money has been spent on developing the peat swamps, and early settlers to the district felt that these organic soils would give a good return for the capital invested for improvements. Unfortunately their optimism was unjustified, and so much money and effort have been uselessly spent in the peat swamps that they have earned a bad name. Successful development of the peat has so far largely depended upon either having an area of higher ground from which to work as a nucleus, or else by "cashing in" on the failure of previous owners.

The magnitude of the problem, together with the fact that the development of the local peat swamps would mean a great deal to the Waikato, made it one of the first to receive the attention of the Soil Fertility Research Station, Hamilton. In order to pursue these studies, an area of approximately 15 acres of the Rukuhia swamp was leased from Mr E. C. Houchen.

The objective of this work has been to establish and maintain a highly productive pasture and to avoid the causes of past failures. The experimental work outlined has been concerned chiefly with the response of pasture and pasture species to various fertilizers and chemicals, and the effects of the treatments on the chemical and physical nature of the peat.

CHARACTERISTICS OF THE PEAT

Taylor¹ has shown how the ridges of gravel deposited by the Waikato River while following its various courses, dammed back the local drainage and resulted in the formation of the three main peat swamps—Moanatuatua, Rukuhia, and Komakorau. The water-logging consequent upon the drainage conditions created, resulted in the accumulation of organic matter which ultimately built up into deep layers of peat. A full description of the botanical nature of the peat has been given by Cranwell². Work on the composition of a peat profile in the Hauraki Plains, where the peat is generally similar to that in the Waikato Basin, was carried out more recently by Harris and Filmer³. The farming of peat land has been described in a general review by Banfield⁴.

In any consideration of the establishment of pasture on the peat, the following points are of major importance: water relationships, consolidation, liability to fire, acidity and supply of plant nutrients. Not all these features have so far received detailed examination at this Station, but it is obvious that they will require investigation in the future.

(a) *Water relationships*.—The problem of the successful reclamation of the peat soils is not purely one of drainage, although drainage is a *sine qua non*, but rather one of maintenance of their water content during both winter and summer at such levels that pastures will grow vigorously. Too deep drainage without adequate consolidation leads to the surface becoming over-dry, and once this state is reached, it is exceedingly difficult to re-wet.

(b) *Consolidation*: As water and air are mutually exclusive, the drainage of the peat is followed by the entrance of air which allows decomposition to commence and consolidation to proceed. To achieve the consolidation of the peat is a prime objective of all developmental work. Factors of importance in effecting consolidation are time of cultivation, type of implement employed, type of grazing animal, and time of grazing.

When peat is well dried out in the summer months no amount of cultivation or trampling will achieve much in the way of consolidation, as the dry surface is then too resilient. True gains in consolidation can therefore only be expected when there is sufficient moisture in the upper layers of the soil. It is unfortunate that in New Zealand there is really no suitable machinery available for working the peat at a time when it is wet and most likely to benefit from the use of heavy implements.

(c) *Liability to Fire*: The readily combustible nature of dry peat is dangerous owing to the constant threat of damage to established pasture, fences and shelter belts in dry weather.

Firing is a useful means of clearing scrub, but fires once started in dry peat are very difficult to put out and are liable to leave depressions which become water-logged in winter.

(d) *Acidity*: The process of peat formation has been accompanied by the development of acid conditions and the raw, unburnt peat has a pH of 3.5 to 4.5. Depending upon the depth of the burn and consequently the amount of ash left behind, burning of the peat in general results in the raising of the pH by approximately one unit.

This acidity of the raw peat is a feature of prime importance in establishing clovers, and therefore in the whole problem of reclamation through the introduction and maintenance of good pasture species.

(e) *Plant Nutrients*: Peat is poorly supplied with the available forms of all the major plant nutrients. The arrested decomposition of the organic matter has left a wide carbon-nitrogen ratio and, in addition to this, the nitrogen is relatively unavailable. The correction of the poor plant nutrient status of the peat is an essential in pasture establishment.

CAUSES OF FAILURE IN PEAT RECLAMATION

At the edges of the peat swamps, the land has been more or less developed for agricultural purposes. In some instances where pasture has been sown, the land has, after several years, reverted to manuka (*Leptospermum scoparium*) and rushes (*Juncus* spp.), and is now unproductive. More commonly, and this applies to extensive areas, development has proceeded to the stage where there is a very poor pasture sward of low productivity, consisting largely of Yorkshire fog (*Holcus lanatus*) or brown top (*Agrostis tenuis*), with very little clover. Since the pasture is of low carrying capacity, relatively few stock may be carried. The whole process of improvement is therefore slow and costly.

The unsuccessful development of the peat in the past has been caused by a variety of factors amongst which are the following:

(a) The inability of the average farmer to secure a sufficiently good pasture early enough to allow him to carry sufficient payable stock to recompense him for money and labour expended.

(b) Piecemeal development of the swamp margins without reference to the ultimate location and direction of drains. Marginal development has been followed by consolidation of the swamp at the edges, thus creating further drainage problems from the raised and untapped areas at the centre of the swamp.

(c) Too big areas have been tackled at a time without adequate fencing or other methods of making controlled grazing in any way feasible.

(d) Lack of knowledge of the fundamental requirements and mineral deficiencies of peat soils.

In spite of the difficulties mentioned, there are many farms on developed peat land which have a very good pasture sward of high productivity. On many of these farms development has taken twenty to thirty years during which the farmer has had a hard financial struggle, particularly in the early stages, when the areas were less productive.

EXPERIMENTAL METHODS

Sampling and preparation of samples

A brass tube of 1½-in diameter was used for taking the peat samples. Each sample consisted of a number of cores, varying from 12 to 24, taken at random. The samples were air-dried, crushed and sieved. Wood and roots retained on a 2-mm sieve were discarded. For the carbon and nitrogen determinations a portion of the sample was re-ground to pass a 90-mesh sieve.

pH.—This was determined in a 1:5 peat-water suspension by means of an electrometer using a glass electrode.

Carbon.—Carbon was determined by Schollenberger's method⁵, using Allison's factor of 86.9 per cent to allow for incomplete combustion and recovery.⁶

Nitrogen.—A micro-Kjeldahl digestion and distillation method, similar to that described for pasture analysis, was used⁷.

Nitrate-Nitrogen.—This was determined in fresh samples by Roller and McKaig's method⁸.

Ammonia-Nitrogen.—Extracts obtained by Crowther and Richardson's modification of Olsen's method⁹ were Nesslerized.

Availability of Nitrogen in Peat

It was mentioned earlier in discussing the general characteristics of the local peats that they have high carbon-nitrogen ratios. The average ratios in samples of peat, taken from various places in the three swamps, in the Waikato basin, are given in Table I. For comparison the table also includes the average ratios for 24 mineral soils representing various soil types from different parts of New Zealand.

TABLE I. - AVERAGE CARBON/NITROGEN RATIOS IN PEAT AND MINERAL SOILS

Origin of sample	Depth of samples (in.)	Number of samples averaged	Average C/N Ratio	Range of C/N Ratio
Undeveloped peat	0-3	5	33	29-37
Undeveloped peat	3-6	9	35	26-52
Semi-improved peat	0-3	2	27	26-28
Improved peat	0-3	3	21	19-24
Burnt peat	0-3	6	14	7-22
Mineral Soils	0-6	24	14	10-25

In view of the high figures for the carbon-nitrogen ratio in peat, a deficiency of available nitrogen would be expected. This was confirmed by determining the mineral nitrogen (ammonia plus nitrate-nitrogen) before and after incubating moist peat for 28 days at approximately 70°F. The results are given in Table II which also includes for comparison, figures for a mineral soil (Te Kowhai loam—a meadow-soil) from the Rukuhia Soil Research Station. The last column of the table gives figures corresponding to Jensen's "Co-efficient of mineralization"¹⁰. This term is defined as the production of mineral nitrogen as a percentage of the initial organic nitrogen (total nitrogen minus mineral nitrogen).

In a study of 46 soils, Jensen obtained coefficients of mineralization ranging from 0.5 to 4.5, the majority of the figures being between 1.5 and 3.0. Three soils from the Rutherglen Experimental Farm, Victoria, were amongst the vigorously nitrifying soils and had coefficients of 3.2 to 4.3. Jensen stated that the "particular aptitude for nitrification which these soils seem to possess may be the explanation why nitrogenous fertilizers have always, at this locality, failed to increase the yield of wheat even when sown on stubble land".

The figures in Table II indicate that the peat is acutely deficient in available nitrogen. Possible methods of rectifying this unfavourable position are:—

(a) Narrowing the carbon-nitrogen ratio by the addition of nitrogenous fertilizers.

(b) Increasing the available nitrogen in the peat through stimulating clover growth. The atmospheric nitrogen fixed by symbiotic bacteria and synthesized into proteins in the clover leaves is eaten by the grazing animal and largely excreted in a form which becomes readily available to plants. The nitrogen in the nodules on the clover roots also augments the nitrogen content of the soil when the nodules break down.

(c) Accelerating decomposition of the peat by oxidizing the carbon, and thus narrowing the carbon-nitrogen ratio.

The experimental work described in the following sections was centred around improving the nitrogen status of the peat by methods (a) and (b) above.

Responses to Nitrogenous Fertilizers

In view of the deficiency of available nitrogen in the peat, it is not surprising that outstanding responses to nitrogenous fertilizers were obtained in field trials. These trials were commenced in 1946 on Mr E. C. Houchen's property on the Rukuhia swamp. The peat here is

TABLE II. MINERAL NITROGEN (EXPRESSED AS PARTS PER MILLION OF OVEN-DRIED MATERIAL) AND CO-EFFICIENT OF MINERALISATION OF PEAT AND A MINERAL SOIL

Description of sample.	Fresh Samples.			After Incubation			Increase in Mineral Nitrogen (p.p.m.)	Total Nitrogen (%)	Coefficient of Mineralisation
	NH ₃ -Nitrogen (p.p.m.)	NO ₃ -Nitrogen (p.p.m.)	Total Mineral Nitrogen (p.p.m.)	NH ₃ -Nitrogen (p.p.m.)	NO ₃ -Nitrogen (p.p.m.)	Total Mineral Nitrogen (p.p.m.)			
Peat - Te Awamutu	3	1	4	37	8	45	41	1.15	0.4
Peat - Ruakura	3	1	4	36	2	38	34	1.00	0.3
Mineral soil - Te Kowhai loam	6	2	8	157	77	234	226	0.51	4.4

TABLE III. - pH, CARBON NITROGEN AND WEIGHT OF PEAT AND MINERAL SOIL

Origin of sample	pH	Carbon * (%)	Nitrogen * (%)	C/N Ratio	Lb. oven dry soil in 0-3 in. per acre
Peat - Rukuhia Expt. area	3.8	51.6	1.45	36	119,000
Semi-improved peat, Ruakura	5.1	26.1	1.00	26	330,000
Te Kowhai loam	5.4	5.8	0.51	11	493,000

* These figures are percentages of material dried at 105°C for 18 hr.

20 to 25 ft deep. The undecomposed, extremely acid, and unconsolidated nature of this peat is indicated by the typical figures for a sample from the 0-3-in layer given in Table III compared with figures from similar samples from a semi-improved consolidated peat on the Komakorau swamp, near Ruakura, and the Te Kowhai loam from the Research Station.

Although the Rukuhia peat experimental area had parallel drains 3 to 4 ft deep, not more than 140 yards apart, the water-table was practically at the surface for several months during the winter of 1947. The preliminary trials included a total of 290 plots topdressed with various combinations of lime, serpentine superphosphate, muriate of potash, ammonium nitrate, minor element mixture (boron, copper, manganese, molybdenum and zinc), limonite, cow manure and top-soil. The trials were laid down in December 1946 in two series. In the first series (Experiment No. 18), the treatments were applied as a topdressing, to pasture consisting largely of Yorkshire fog with small patches of white clover (*Trifolium repens*) and *Lotus major* which had been sown the previous autumn.

The second series (Experiment No. 18A) was on an adjacent paddock previously in manuka. In this series, the area was disced, the manuka removed, and the treatments incorporated with the top few inches of peat before the area was sown with a mixture of Yorkshire fog, perennial ryegrass (*Lolium perenne*), and *Lotus major*.

The most outstanding result of these trials, judged by eye observations of the plots, was a substantial but relatively short-lived increase in the growth of the grasses on all plots topdressed with ammonium nitrate, irrespective of other fertilizers used. In these trials, ammonium nitrate was applied at three rates—1 cwt, 5 cwt, and 20 cwt per acre. The general layout of some of the plots in Experiment No. 18 and the response to ammonium nitrate is shown in the accompanying photograph (Fig. 2).

In a subsequent trial on pasture plots both ammonium sulphate and sodium nitrate gave similar responses to ammonium nitrate when equal rates of nitrogen were applied.

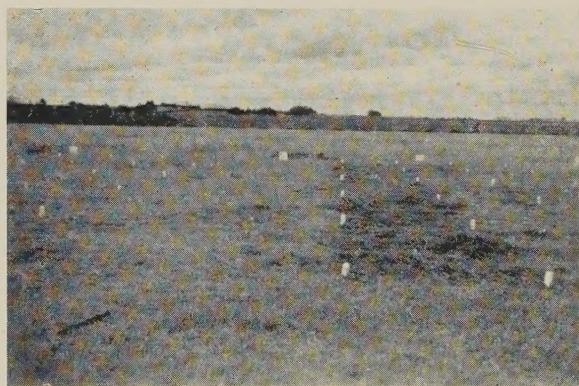


FIG. 2.—A general view of some of the plots in experiment No. 18, taken four months after the fertilizers were applied. The vigorous growth of grasses on the plots treated with 20 cwt per acre of ammonium nitrate, is shown in the dark row to the right of the centre.

In all these experiments, it was noted that when no phosphatic fertilizer was applied the flush growth of pasture resulting from the applications of nitrogenous fertilizers had the purplish tinge associated with phosphorous deficiency,¹¹ but this coloration was absent on similar plots receiving serpentine superphosphate.

In his investigations on plant tissue tests for the diagnosis of soil deficiencies, Mr K. J. McNaught of this Station examined samples from some of these plots. The results by the Carolus method¹² from samples from plots in Experiment No. 18 (Table IV) confirm the phosphorus deficiency. The poor growth on untreated plots had a high phosphorus level, but this dropped to a deficiency level in the flush growth following heavy nitrogen topdressing. When phosphatic fertilizer is also applied the high phosphorus level in the tissue is maintained. Similarly the flush growth accentuated the potash deficiency in the absence of potash fertilizers.

In these experiments, heavy applications of ammonium nitrate tended to eliminate legumes from the pasture sward. On the plots receiving 20 cwt per acre there were no clovers visible within four months of the applications, and there was only very little re-entry of clovers 18 months after the applications.

A striking feature of the heavy nitrogenous dressings was that within a few months perennial ryegrass appreciably replaced Yorkshire fog in the sward, whereas on the corresponding no-nitrogen plots it was difficult to find any ryegrass.

Spectacular responses to 2 cwt and 5 cwt per acre of ammonium nitrate were also obtained on a crop of young oats on fairly raw peat at the edge of the Moanatuatua swamp near Te Awamutu, and on established pasture on semi-improved peat on Mr C. J. Brooke's property at Ruakura. The peat from the latter area is referred to in Tables II and III.

In view of these outstanding responses, a trial was commenced in February 1947 to determine whether the good pasture growth initiated by nitrogenous fertilizers could be maintained under controlled grazing by returning the pasture produced to the soil through the grazing animals. Approximately an acre of the paddock in which Experiment No. 18 was situated was enclosed and strips topdressed with ammonium nitrate at 1, 3, and 5 cwt per acre, and half the area re-topdressed in June. Although the pasture responded immediately to the nitrogenous topdressings, 12 months later the pasture in the enclosure was little better than that outside. The controlled grazing in the enclosure did, however, keep the land clear of manuka and rushes, whereas in the surrounding paddock the scrub grew about two feet high during the course of this trial.

Although the ammonium nitrate applications in some of these experiments were made during a very dry period in the autumn when all other pasture on the peat was suffering severely from lack of moisture, the pasture on the nitrogen treated plots was green and vigorously growing. This suggests that adequate nitrogen minimizes the effect of water shortage. In this respect it is of interest to note that Collison *et al.*¹³ stated that nitrogenous fertilizers enabled crops to produce dry matter with a smaller water utilization.

*Establishing and Maintaining Clovers on Peat**Effect of pH*

In the Yorkshire-fog-dominant pasture, on which Experiment No. 18 was laid down, it was noted that white clover grew in patches only. The peat below the clover patches had a pH of approximately 5, whereas peat from non-clover areas had a pH closer to 4. The appearance of the peat suggested that the difference in pH was the result of the former areas being burnt. In subsequent experiments both in the field and in pots in the glass-house, the effect of raising the pH from about 4.0 to 5.0 by either burning or liming was examined on white clover, subterranean clover (*Trifolium subterraneum*) and *Lotus major*. The clovers germinated equally well with all treatments, but in the field trial the seedlings on the peat at pH 4.0 all died within a few months, whereas fair growth was obtained at the higher pH. The seedlings in the pots at pH 4.0 survived, but the growth was very poor compared with that at pH 5.0. In both experiments liming, which was at the rate of approximately one ton of calcium carbonate per acre, facilitated the establishment of clovers better than burning.

A further small scale field trial (Experiment No. 18E) on the Rukuhia experimental area was designed to determine the effect of variations in pH and manurial treatment on clover growth. The 0 to 3-in layer of the unlimed peat was approximately pH 4.0 and it was aimed to obtain, by appropriate liming, successive strips with the pH increasing by 0.5 unit over the range 4.0 to 7.0. Hydrated lime was applied in May 1947 to six strips, each 16 yd long and 1 yd wide, of cultivated peat, while another strip was left unlimed. Eight cross rows each two yards wide were treated with fertilizers and chemicals.

The lime and the fertilizers were worked into the top few inches of peat, and a week later white clover was planted over the whole area, the individual plants 9 in apart. Periodical determinations were made of the pH of the peat and observations were made of the growth of the clover plants.

The effects of the applications of lime, phosphate and potash on the growth of white clover may be summarized as follows:—

(a) On unlimed acid peat, growth was negligible even when appreciable quantities of phosphate and potash were applied.

(b) In the absence of phosphate and potash, growth was poor, but increased with successive increments of lime.

(c) In the presence of phosphate and potash, liming up to approximately pH 4.5 (equivalent to 2 tons of calcium carbonate) increased growth, but liming to pH 5.0 (equivalent to 4 tons of calcium carbonate) or higher decreased growth.

The above responses to lime up to pH 4.5 are probably due in part to the increase in the supplies of calcium, since this peat is low in calcium.

This lack of response to lime above pH 5.0 was confirmed by a top-dressing experiment on the semi-improved peat at Ruakura. On this peat (pH 5.1), pasture plots topdressed with phosphate and potash did not respond to 1 ton of lime. Some observers even considered that the plots without lime were better than the limed plots. Analyses showed that this peat was adequately supplied with calcium.

The adverse effect of heavy liming is in agreement with the results obtained in the development of peat areas in Germany. Brune¹⁴ stated that apart from economic aspects, it is a great mistake to use too much lime in the development of the high moor (acid) peats, because even with lime-demanding crops such as legumes the upper limit of lime that the plants can withstand on high moor is much lower than one would expect, according to the acidity of the soil.

One frequently quoted explanation of the injurious effect of over-liming acid peats, based on findings by Arnd^{15, 16} is that heavy liming stimulates nitrification, and that under the anaerobic conditions of the peat, denitrification occurs with subsequent loss of nitrogen.

Determinations of total nitrogen in samples of peat from the Experiment No. 18E did not indicate any significant loss of total nitrogen on the heavily limed plots. Available (mineral) nitrogen was not determined, and there may have been some loss in this respect, but it is unlikely that this could have accounted for the depression in growth with high liming, since the clover plants were adequately supplied with nodules. It is possible, however, that denitrification occurred, and that the decreased growth was due to the concentration of nitrite-nitrogen reaching toxic levels.

TABLE IV. TISSUE TESTS ON YORKSHIRE FOG

Fertilizer Application 12/12/46	Date Sampled	P.p.m. Wet Tissue		
		NO ₂ -Nitrogen	Phosphorus	Potassium (as K ₂ O)
Control	8/1/47	5	410	3100
5cwt ammonium nitrate	"	368	125	2500
5cwt ammonium nitrate plus L.P.K.	"	213	340	6000
Control	6/3/47	8	380	3200
20cwt ammonium nitrate	"	683	104	1400
20cwt ammonium nitrate plus L.P.K.	"	260	249	4200

Injurious effects of over-liming acid mineral soils have been frequently reported and have been attributed to various causes. For instance, the reasons given in the literature quoted by Midgley¹⁷, and Lynd and Turk¹⁸ include excess of soluble calcium, nitrite-nitrogen and ammonia-nitrogen, and deficiencies of boron, manganese, phosphorus and potassium. However, the findings regarding mineral soils may not apply to organic soils in view of the differences between the two types.

Effect on Clover Growth of Topdressing with Phosphate and Potash

In Experiment No. 18 the original Yorkshire-fog-dominant pasture changed within 12 months to a clover-dominant sward as a result of topdressing with a mixture of 2 tons of carbonate of lime, 10 cwt serpentine superphosphate, and 2½ cwt muriate of potash per acre. Further experiments were conducted on Mr Brooke's property at Ruakura to determine the relative importance of phosphate and potash in stimulating clover growth, and to determine the minimum quantities of each required to give good results.

In Experiment No. 18F, strips 2 yd wide and 20 yd long were treated in June 1947 with fertilizers at the following rates per acre: -

Strip 1: 10 cwt serpentine superphosphate.

„ 2: 2 cwt serpentine superphosphate.

„ 3: Control (no manure).

„ 4: 1 cwt muriate of potash.

„ 5: 5 cwt muriate of potash.

„ 6: 10 cwt serpentine superphosphate and 5 cwt muriate of potash.

„ 7: 2 cwt serpentine superphosphate and 1 cwt muriate of potash.

„ 8: Same as Strip 6 but plus 1 ton of lime.

„ 9: Control (no manure).

The pasture was originally chiefly brown-top with some *Paspalum* (*Paspalum dilatatum*), flat weeds and a sprinkling of clovers. The efficiency of the various topdressing treatments was judged by their effect on the clover growth since, as explained later, clover growth is considered the key to the development of peat. The outstanding result of this experiment was the prolific growth of white clover on Strips Nos. 6 and 8, i.e. those receiving 10 cwt serpentine superphosphate and 5 cwt muriate of potash per acre.

This experiment was designed for observation only and not for cutting and weighing the yields. However, in order to obtain an indication of the response to the topdressing, cuts were taken from Plots Nos. 3 and 6 on 10 December, three months after the paddock was closed for hay. The results given in Table V show that the topdressing resulted in a big increase in yield.

The extent to which this increase in growth as a result of topdressing was due to increase of white clover in the sward, is indicated by the data in Table VI which give the result of botanical dissections of samples of pasture from the control strips and from the topdressed Strips Nos. 6 and 8.

The other treatments in this experiment, namely phosphate or potash only and a mixture of 2 cwt serpentine superphosphate and 1 cwt of muriate of potash per acre, were a little, but not much, better than the control strips.

In order to obtain further data regarding the minimum quantities of phosphate and potash required to give good results, another experiment (No. 18H) was commenced in September 1947 in the same paddock as

TABLE V. YIELD OF PASTURE FROM PLOTS

Plot	Wet weight of pasture per cut (lb)	Dry Matter (%)	Yield of dry matter per cut (lb)	Yield of dry matter per acre (lb)
<u>Plot No. 6</u> 10cwt serpentine super and 5cwt muriate of potash	36.8	15.5	5.7	2760
<u>Plot No. 3</u> Control	11.1	22.8	2.5	1210

Experiment 18F. Four strips, each 4 yd by 16 yd, were treated with serpentine superphosphate at 2 cwt, 4 cwt, 7 cwt, and 10 cwt per acre respectively. Four cross rows of similar dimensions were topdressed with $\frac{1}{2}$ cwt, 1 cwt, 2 cwt, and 5 cwt muriate of potash per acre respectively.

TABLE VI. BOTANICAL COMPOSITION OF PASTURE
EXPRESSED AS PERCENTAGE OF OVEN-DRIED MATERIAL

Plot No.	Topdressing per acre	Grasses (%)	Clovers (%)	Other Species (%)
3	Control	55	17	28
9	"	40	21	39
	Average for control plots	47	19	34
6	10cwt serp.super 5cwt mur. of potash	22	51	27
8	10cwt serp.super 5 " mur. of potash 20 " lime	31	64	5
	Average for topdressed plots	27	57	16

The paddock was closed for hay, and by December there was a most outstanding increase in growth, particularly of white clover, on the plots receiving the largest applications of both fertilizers. The growth was 8 to 10 in high compared with about 3 in in the untreated paddock. In the strip which received 10 cwt serpentine superphosphate the growth increased with each successive increment of potash. The increase in response between 2 cwt and 5 cwt of potash was considerably more than the increase from 1 cwt to 2 cwt. Similarly in the row which received 5 cwt of muriate of potash the growth increased with each increment of superphosphate. The difference in this case between 7 cwt and 10 cwt was not very marked.

The heaviest application of one fertilizer gave less response than an intermediate rate when crossed with the lowest rates of the other fertilizer, for example, 7 cwt of serpentine superphosphate was better than 10 cwt when crossed with either $\frac{1}{2}$ cwt or 1 cwt of muriate of potash. Similarly 2 cwt of potash was better than 5 cwt when crossed with 2 cwt serpentine superphosphate.

Although the best results were obtained from the combination of both fertilizers at the highest rates used, very good results were obtained from the mixture of 7 cwt serpentine superphosphate and 2 cwt muriate of potash. The response from this mixture would have been regarded as excellent if it had not been surpassed by the response from the still higher rates of fertilizers. The results in general suggest that the minimum quantities of the two fertilizers required to give good results are 4 to 7 cwt of serpentine superphosphate, and 2 to 5 cwt of muriate of potash.

DISCUSSION AND CONCLUSIONS

From a consideration of all the experimental data, which includes the results of Mr McNaught's tissue tests, analyses of peat, and observations on over 400 plots treated with various combinations of fertilizers, it is considered that the growth of grasses on peat land, and in particular high-fertility-demanding species such as perennial ryegrass, is primarily limited by the deficiency of available nitrogen. Low pH and deficiencies of potassium, phosphorus, and calcium, are considered to be secondary limiting factors. The local peat therefore contrasts strikingly with the Everglades peat in Florida, in which nitrification was so active that figures of up to 400 p.p.m. of nitrate-nitrogen were found¹⁹ and on which no response to nitrogenous fertilizers was obtained²⁰.

Our experimental work has shown that, notwithstanding this nitrogen deficiency, nitrogenous fertilizers are not the key to the successful development of the peat because their effect is too short-lived; they depress clover growth and their use does not obviate the necessity to add other fertilizers to remedy the mineral deficiencies in the peat.

The good clover growth on the experimental plots which resulted from the applications of potassic and phosphoric fertilizers, together with lime on very acid areas, suggests that symbiotic fixation satisfactorily overcomes the nitrogen deficiency so far as clovers are concerned.

These results suggest that, by producing good clover growth by appropriate topdressing, it should be possible, with the aid of the grazing animal, to increase the available nitrogen in the peat sufficiently to support a good mixed sward of perennial ryegrass and white clover. A striking increase in perennial ryegrass is already taking place on the plots which had become clover dominant as a result of topdressing.

Although the importance of clovers in grassland farming is well known, the particular value of clovers on peat due to the acute nitrogen deficiency has not been adequately recognized previously. Failure to appreciate this has probably contributed to the slow development of the peat in the past.

In general, conditions suitable for plant growth are favourable for the microflora in the soil. The topdressing required to obtain good clover growth on the peat probably makes conditions more favourable for the micro-organisms. Thus, it is hoped that a long-term benefit from the topdressing will be the accelerated decomposition of the peat with subsequent narrowing of the carbon-nitrogen ratio, increase in the available nitrogen, and improvement in consolidation and drainage.

In this discussion it is assumed that, as a pre-requisite to sowing pasture, the area would be appropriately drained. Obviously, topdressing would not give good results on areas either continually waterlogged or grossly overdrained to the extent that the moisture content is below wilting point for long periods. However, the good results obtained on the Rukuhia experimental area which was waterlogged for several months during the winter, show that topdressing can be effective even though the drainage is far from ideal.

The results indicate that on peat land the quantities of potassium and phosphorus available to plants have to be increased appreciably before good clover growth can be expected. This increase may be effected by means other than heavy applications of fertilizers. Many areas have

been successfully developed over a long period of years by feeding out hay on the peat. In addition to advantages resulting from the consolidation due to heavy stocking, the feeding out of hay is in effect a means of gradually building up the potassium and phosphorus in the peat.

Repeated burning of the peat is another practice commonly used in development. This gradually concentrates the mineral content so that in time the surface layers have sufficient minerals for good clover growth. However, burning has its disadvantages. The best burns occur in particularly dry years when the water-table is abnormally low. When the water-table subsequently returns to normal, the area is liable to be water-logged. Burnt peat tends to dry out badly due to loss of water-holding capacity as a result of the destruction of the organic matter, and pastures on deep burns are particularly liable to suffer in both wet and dry seasons.

It appears from our results, and from observations in general that the extent of mineral deficiency in peat has not been realized. The rates of topdressing on the experimental plots found to be necessary to obtain good clover growth are very much higher than those normally used in practice, but the pasture responses were correspondingly greater than those usually obtained. If, however, it can be shown that these heavy applications are required for the initial period only, and that good growth can be maintained by subsequently topdressing at normal rates, the development of peat land along these lines may eventually prove to be the most economical method. The critical factor is the rate of annual topdressing required to maintain good growth, and this is the subject of current experimental work.

In conclusion, it is stressed that the experimental work described in this article is of a preliminary nature only. It covers the exploratory stages in which a broad field has been covered in an endeavour to determine the main factors limiting growth of pasture on peat. There is still much more to be learnt regarding the most economical means of remedying these principal limiting factors and the interaction of other factors such as drainage, consolidation, and decomposition. However, the results to date have been encouraging, and suggest that a more optimistic view may be taken than hitherto with regard to the possibilities of developing the peat swamps in the Waikato district.

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MICROSCOPIC EXAMINATION OF THE SECTIONED STEM OF LINEN FLAX (*Linum usitatissimum*)

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PART I: MICROSCOPIC ANALYSIS AS A MEANS OF PRE- DETERMINING YIELD OF SCUTCHED FIBRE

Summary

The total percentage of fibre in a transverse section of a linen flax stem was calculated in the manner given below. Values from ten stems of a crop were averaged and the resulting percentage was correlated with that obtained by retting and scutching 25 lb. of the same crop. Ten crops were dealt with and a correlation attempted, but statistical analysis revealed that either the variance between calculated total percentage and the fibre content value obtained by retting and scutching was too great, or the number of stems sectioned and analyzed was too small for this method to prove satisfactory in the predetermination of fibre yield.

The microscopic analysis did, however, show that the variation in percentages within crops was smaller than that between different crops, the correlation being highly significant. Although it would be impossible, using only ten stems, to predict the fibre percentage of any particular crop with sufficient accuracy for commercial valuation, the method, nevertheless, should be very useful in connection with breeding and selection, and especially with single plant selections.

SCOPE AND PURPOSE OF INVESTIGATION

This report covers an investigation into the possibility of determining the yield of scutched fibre by a microscopic examination of transverse sections of a number of stems taken at random from sample sheaves, the actual yields of which were later determined at the Linen Flax Research Laboratory.

Ten crops of the 1946-47 season were chosen whose yields, determined by retting and turbine scutching, ranged from 3 per cent to nearly 17 per cent. The total percentage of fibre present in a transverse section of a stem was calculated from measurements taken from projections of these sections on photographic paper. A correlation was then attempted between the calculated total percentage of fibre present in the straws and that obtained by retting and scutching the sample sheaves.

The necessity for this investigation arose from the fact that the flax crop is purchased in New Zealand on a fibre-content basis which necessitates the sampling of each crop as it is delivered to the factory. This sample is processed to determine the amount to be paid to the grower. It was thought worthwhile to investigate the possibility of predetermining the yield of scutched flax, by the methods described in this report, in the hope that such a method might at least support, if not replace, the present costly and laborious method of processing and scutching each of the hundreds of samples forwarded each year for fibre determinations.

It was thought that even if this purpose were not attained, sectioning and microscopic examinations might serve other purposes. For example, crops which fail to reach a certain minimum fibre content are rejected in the field, but identification of such crops is difficult and might lead to serious mistakes. It was considered that some guidance might be possible to those responsible for acceptance and rejection in the field, if representative samples were drawn just prior to harvest and examined microscopically.

As a final objective, it was felt that a rapid method of microscopic analysis might well be of service to those undertaking the selection and breeding of flax.

EXPERIMENTAL PROCEDURE

For a microscopic analysis to be of practical use in the determination of fibre yield, obviously only a few sample stems must suffice because of the limitations to the numbers that can be sectioned and analysed. As only a small hand-microtome was available for sectioning, it was not practicable in the time available to section more than 10 stems from each crop. These stems were taken at random from the sample sheaves, the fibre content of which was later determined by retting and turbine scutching. Thus from each of ten crops which had been selected from the flax-producing districts of the South Island, ten sample stems were sectioned and analyzed.

Preparation of Sections:

This procedure was identical with that described by Raeside and Mason (1). The portion of stem selected for sectioning was a two-inch length cut off from immediately below the midpoint between the first branch of the inflorescence and the stem at ground level. The fixative used was formo-acetic alcohol. A small length of the fixed flax stem was inserted in a piece of elder pith which was made to fit the head of the microtome. After moistening the pith to ensure a firm fit, the sections were cut off with a razor and mounted in a solution consisting of equal parts by volume of glycerine and water.

Method of Projecting Sections on to Photographic Paper:

For easy measurement and to serve as a means of recording the appearance of the ten sectioned stems of each crop, a method was employed of projecting the microscopic image directly on to photographic paper.

The microscope was mounted horizontally on a sliding platform at a sufficient distance from the focussing screen to form an image of convenient size for 10 in. x 12 in. photographic paper held in a frame on the screen. A $\frac{2}{3}$ in. objective and a x8 ocular were the lenses normally used, and the microscope was fitted with an iris diaphragm and a high power substage Abbé condenser. The microscope lamp used was a 110 V. concentrated filament type, but during exposure the voltage was reduced to 80 V. to allow a longer exposure. The beam was projected directly through the microscope without use of a mirror. Times of exposure varied from 10 to 15 sec. owing to the slight differences in section thickness.

When lamp, condenser and diaphragm had been arranged to give maximum definition in the focussed image projected on the screen, the microscope lamp was extinguished, leaving only an orange dark-room lamp for illumination. A small portion of normal glossy print paper was fitted within the paper-frame and the microscope lamp switched

on for a trial exposure. When the exposure time had been determined in this manner, a 10 in. x 12 in. sheet of the same paper was placed in the frame and the exposure completed. Developing and fixing was as recommended by the manufacturers of the paper.

This afforded a quick method of recording the microscopic appearance of the sections, and although the projections were negative, they were quite satisfactory for making all required measurements, and for counting fibres. Projections were made of ten sample stems from each of the ten selected crops.

Calculation of Magnification of each Projection:

The magnification of each projection was calculated from the formula

$$M = m_{obj.} \times m_{oc.} \times \frac{d}{10}$$

where M = total magnification

$m_{obj.}$ = magnification of objective

$m_{oc.}$ = " " eyepiece

d = distance in inches from top of eyepiece to photographic paper fixed on screen.

Magnifications ranged from 73 to 390 diameters, the average being at about 200 diameters.

Measurements of the diameters of projected images were made, from which the actual stem diameters were readily calculated.

Determination of Total Fibre Percentage in a Transverse Section:

The method adopted for this calculation was devised by A. O. Gidall of the Linen Flax Research Laboratory. Although slow, it proved to be accurate. From each projection a tracing was made of the outlines of the stem, pith and fibre bundles. The complete tracing of the stem was then cut out and weighed, after which the pith tracing was cut out from the centre, and the weight obtained of the tracing of the stem minus the pith. Next, the fibre bundle tracings were carefully cut out and weighed. The weights of the pith tracing and the remainder after cutting out the fibre bundles were also recorded as checks.

From these measurements, the weight of the fibre bundle tracings divided by the weight of the stem minus pith tracing gave a ratio which represented the total percentage of fibre present in the transverse section of the stem. The ratio of $\frac{\text{weight of fibre bundle tracing}}{\text{weight of stem minus pith tracing}}$ gives the relative *area* occupied by the fibre in the transverse section. The percentage of stem area occupied by fibre is, of course, not identical with the true fibre percentage by weight owing to the differences in specific gravity of the various plant tissues, but is satisfactory when comparing one stem section with another.

A typical calculation is given below:—

Weight of whole stem (tracing) including pith	=	1.900 g.
" " stem minus weight of pith	.. =	0.920 "
" " pith	.. =	0.980 "
" " (stem-pith-fibre bundles)	.. =	0.705 "
" " fibre bundles	.. =	0.215 "
Ratio of $\frac{\text{weight of fibre bundles}}{\text{weight of stem-pith}}$	.. =	$\frac{0.215}{0.920}$
		= 23.37 per cent

i.e., total amount of fibre in this transverse section is 23.37 per cent of the area.

This percentage was known as the calculated total percentage of the fibre in the stem. The average percentage for the ten stems of each crop was known as the calculated average total percentage of fibre in that crop, referred to for convenience as "total fibre percentage," and distinct from the percentage obtained by retting and scutching 25 lb. of the same straw. This latter value was known as the actual extracted percentage of scutched fibre, referred to hereafter as "actual yield."

The total fibre percentage per stem for each of the ten crops is listed below in Table I together with the average percentage for each crop.

TABLE I. CALCULATED TOTAL FIBRE PERCENTAGES PER STEM
FOR EACH OF THE TEN CROPS

Crop No.	MV40A.	FL27A.	MK20.	MK15B.	FL27B.	MV27A.	WT50.	FL8.	MV28.	FL27C.
Calculated	15.0	20.0	16.5	15.0	20.8	16.5	16.2	16.5	16.5	17.7
Fibre per-	15.1	21.1	17.8	15.2	22.1	16.5	18.2	18.5	19.4	19.0
centages of	15.3	21.5	18.1	16.0	22.1	16.6	19.3	19.3	20.2	20.8
each stem.	15.5	22.8	18.1	16.4	22.6	17.9	20.0	20.3	20.8	21.6
	15.7	23.6	19.6	17.0	22.6	18.8	20.3	20.4	21.5	22.6
	15.8	23.6	20.3	18.2	22.8	19.0	20.6	20.6	22.7	22.4
	16.3	24.6	20.5	18.3	23.3	19.2	21.0	21.6	23.4	22.8
	16.9	25.0	20.9	18.5	24.3	20.0	21.0	22.9	23.9	23.9
	18.2	25.9	21.1	19.6	25.0	20.4	21.2	23.6	24.0	26.0
	18.6	27.2	23.7	20.0	27.3	21.3	22.2	24.3	24.1	27.3
Average.	16.2	23.5	19.7	17.4	23.3	18.6	20.0	20.8	21.6	22.4

The calculated average total fibre percentages of ten crops were listed in ascending order and compared with the corresponding actual yields. It was observed that there was a slight correlation which was not, however, sufficiently significant for the calculated figure to serve as a basis for the prediction of the actual yield.

TABLE II. CALCULATED TOTAL FIBRE PERCENTAGE FOR EACH CROP
COMPARED WITH ACTUAL EXTRACTED FIBRE

Crop No.	MV40A.	MK15B.	MV27A.	MK20.	WT50.	FL8.	MV28.	FL27C.	FL27B.	FL27A.
Calculated Fibre Per cent.	16.2	17.4	18.6	19.7	20.0	20.8	21.6	22.4	23.3	23.5
Actual Ex- tracted Per cent.	5.9	3.5	12.9	16.8	8.8	10.6	7.3	13.1	12.7	11.3

"Calculated total percentage" is a value obtained from an average of ten stems which are not sufficient to be truly representative of a crop containing some millions of stems. The yield of scutched fibre resulting from retting and turbine scutching is a more practical measure, since it takes into account such factors as length of straw, amount of weed present, condition of the straw, etc., which are not taken into account in the calculated total percentage. If calculated percentage increased progressively with actual yield, the graph would take the form of a straight line, its slope determined by the nature of the increasing fibre percentage. However, it can be seen from Fig. 1 that deviations from the ideal are large, although there is some tendency for calculated yield to increase with experimental yield.

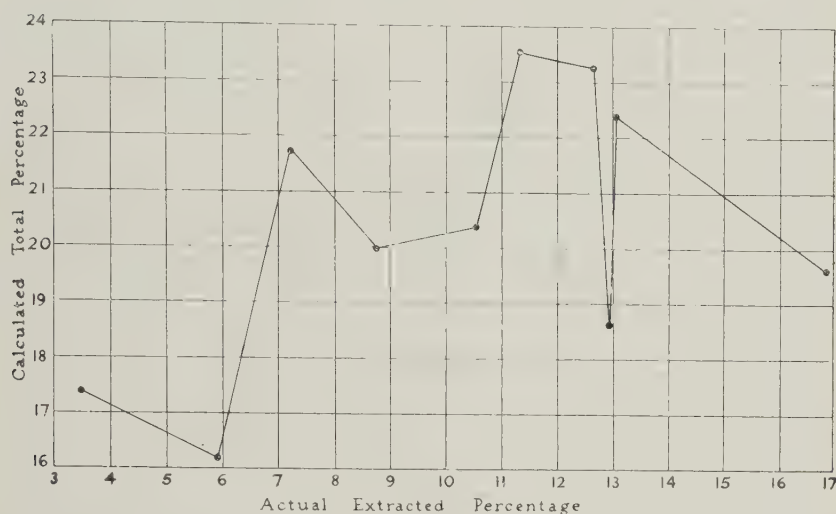


FIG. 1.—Graph showing relationship between calculated fibre percentage and actual extracted fibre percentage.

It is possible that if a large number of stems from each crop were analyzed, these deviations would be minimized and calculated yield would increase steadily with actual yield. If such were the case, it might be possible to predict fibre yield by microscopic analysis, but the number of stems requiring to be analyzed would make this method commercially impracticable.

One observation which might prove to be of considerable importance was made when the ratio $\frac{\text{diameter of stem}}{\text{diameter of pith}}$ (referred to hereafter as $\frac{s}{p}$) for each of the 100 stems was compared and correlated with the corresponding calculated fibre percentage. It was noted that the greater the value of this ratio the smaller was the calculated fibre percentage. Thus, a stem with a comparatively small pith area and a correspondingly large amount of xylem could be expected to have a small percentage of total fibre present. This view is one which had been regarded as probably true but as far as is known this is the first evidence which supports this theory.

TABLE III. ANALYSIS OF VARIANCE AND COVARIANCE ON LINEN FLAX SECTIONS

	d.f.	M.S.(x ²).	M.(xy).	M.S.(y ²).
Between crops	9	.614	—1.97	46.7
Within crops	79	.031	— .129	4.77
Total	88			

$$x = \frac{s}{p} \quad y = \text{Per cent. fibre (calculated)}$$

$$\text{Correlation within crops } \rho_{xy} = -.33$$

$$\text{Correlation between crops } \rho_{xy} = -.35$$

The figures for the ten stems of the ten crops were statistically analyzed and the constancy of the value $\frac{S}{P}$ within any particular crop was found to be significant. When the values of $\frac{S}{P}$ for the 100 stems were plotted against the calculated fibre percentages there was a significant correlation.

The results show that there is a much closer correlation within crops than between crops, and this method, involving the calculation of the ratio $\frac{\text{stem diameter}}{\text{pith diameter}}$ and its correlation with the calculated fibre percentage of that stem, should be of importance in the selection and breeding of linen flax.

CONCLUSION

The results of this investigation show that the microscopic analysis of ten stems from each crop is not sufficient to provide a practical basis on which to predict fibre yield. Total fibre content, as calculated from measurements taken from projections of the sections on photographic paper, is not a practical term, as it does not take into account such factors as condition of the straw, weathering, etc., which can greatly affect the actual yield at the factory. It has been shown that very many stems would need to be examined microscopically before this method could be considered a reliable one for the prediction of fibre yield, but this condition makes the method unwieldy and of no practical use.

The investigation has shown, however, that there is a distinct difference in the appearance of transverse sections of stems from high-yielding and low-yielding crops. Before a crop is accepted it must give a yield of not lower than 7 per cent of fibre on crop weight, this determination being made by retting and turbine scutching at the Research Centre. From the figures of total fibre content listed in Table I, it can be seen that the two reject crops, MV.40A. and MK.15B. the yields of which were 5.9 per cent and 3.5 per cent respectively (determined by retting and scutching), gave the lowest values of calculated total fibre content. Also, projections of the stems of these crops showed that they all possessed more xylem than stems of high yielding crops. This method of microscopic examination, therefore, although not reliable enough to predict fibre yield to an accurate figure, may be used, nevertheless, as a guide by those responsible for acceptance or rejection of crops in the field.

Finally, work on the ratio $\frac{\text{stem diameter}}{\text{pith diameter}}$ and its correlation with calculated total fibre content, has revealed that for these two values there is a greater correlation within crops than between crops, and as plant breeders are concerned with the total fibre content of each pure line they breed, this observation should be of service to them.

PART II: EXAMINATION OF QUALITY FACTORS AND THEIR CORRELATION WITH COMMERCIAL GRADING

Summary

Six factors were selected as influencing fibre quality, and were used as a basis of analysis by means of which the microscopic appearance of flax sections was correlated with the grade of the processed fibre. Good correlation of the derived "fibre quality index number" and commercial grade was found, although further confirmation of these results is necessary owing to the small number of stems analysed from each crop.

Good correlation was also found between grade and the fibre extraction percentage, i.e. the actual yield of fibre expressed as a percentage of the total calculated fibre content of a crop. These observations emphasize the close interrelationship between grade and fibre yield, and the impossibility of obtaining a true understanding of one without a study of the other.

INTRODUCTION

As at the present time there is no method of pre-determining the quality of linen flax fibre, investigations were undertaken to discover whether there is any connection between the quality of such fibre and certain microscopic characters. In these investigations the method of analysis used by Raeside and Mason¹ was followed with the modification that layering, fibre size and number of fibres per unit area of stem (characters included in the fibre index number derived by Raeside and Mason) were omitted and a new method of rating for lignification was substituted.

The six characters listed below were selected as most likely to influence fibre quality, and these were each divided into three or four grades to which numerical ratings were allotted, the size of the number being in accordance with the degree of development of that particular character, and with the effect it was thought to have on fibre quality. The sum of the numbers of the individual characters of each stem was designated the "fibre-quality index number", from which the average fibre-quality index number for each crop was calculated.

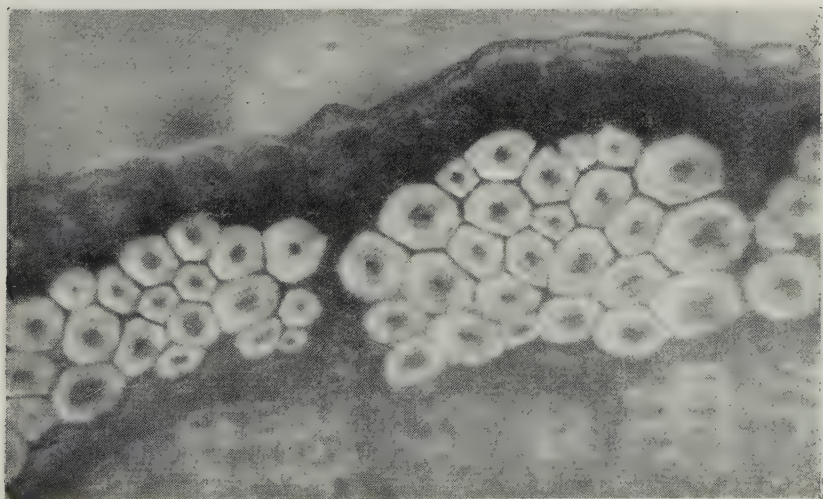


FIG. 2.—Good, thick walled fibres of linen flax in transverse section. X 450 (approx.)

The system of rating the various quality factors, which is a modified version of that used by Raeside and Mason,¹ is given below:—

QUALITY FACTOR. RATING

Bundle Shape

In determining the rating to be given for bundle shape it was necessary to take into consideration spacing of the bundles.

Regular in shape	1
Moderately irregular in shape	2
Very irregular in shape	3
Coalescing into one continuous ring	4

Evenness of Fibre Shape within Bundle

Even in shape and lying evenly in rows	1
Uneven in shape and size	2
Divided into two zones, the inner ones usually showing poor shape, very often squashed or folded	3

Fibre Shape

Polygonal or round	1
Slightly flattened	2
Squashed	3
Folded, i.e. the cell wall folded inwards and the shape of the cell wall distorted	4

Wall Thickness of Fibre Cells

Thick	1
Medium	2
Medium-thin	3
Thin	4

Different wall thicknesses are illustrated in Figs. 2 and 4.

Lignification

Number of lignified cells expressed as percentage of total fibre cells indicates the degree of lignification.

Less than 0.5	1
0.5 - 2.5	2
2.5 - 4.5	3
4.5 - 6.5	4
6.5 - 10.0	5

Lignified cells are apparent in Fig. 3.

Poor Fibres

Poor fibres expressed as percentage of total fibres.

0 - 1	1
1 - 3	2
3 - 10	3
More than 10	4

From the above it can be seen that a stem exhibiting the characteristics believed to be associated with high grade would be here allotted a quality index number of low numerical value.

The results set down in Table IV below show the correlation between quality index numbers and grade; in the ten crops analysed, those yielding fibre of the lowest grade were those which were allotted the highest index numbers on microscopic analysis.

TABLE IV. CORRELATION OF QUALITY INDEX NUMBER WITH COMMERCIAL GRADE

Crop.	Bundle Shape.	Even-ness within Bundle.	Fibre Shape.	Wall Thick-ness.	Lignifi-cation.	Percen-tage Poor Fibres.	Fibre Index Number.	Grade.
MK.20	2	2	2	2	1	1	10	C C C B B B A A
FL.27C	3	1	1	1	1	1	8	
FL.27B	2	1	1	2	3	1	10	
MV.27A	2	1	2	2	3	1	11	
FL.27A	3	1	1	1	2	1	9	B B B A
FL. 8	2	1	2	2	2	2	11	
WT.50	2	1	2	1	3	1	10	
MV.28	3	1	1	2	4	1	12	
MV.40A	2	2	2	2	4	1	13	A A
MK.15B	3	2	1	2	3	3	14	

Although fairly good correlation was obtained, the results should not be regarded as conclusive as it is fully realized that the size of the sample examined is in no way proportional to the size of the crop. Many factors may play a part in bringing about the lack of correlation found by previous workers between "microscopic quality factors" and grade. Examples of such factors are the deterioration of the fibre quality between time of taking the sample for sectioning and time of processing the bulk sheaf, lack of knowledge of the relevant importance of the characters on which the quality index number is based, and the uncertainty of grading which is necessarily dependent on the human factor. Much more material would need to be analysed for the results here obtained to be confirmed.

It was found, also, that the percentage of lignified cells in the stems could be correlated with the grade of the crop from which the stems were taken. Poor quality flax possessed quite a high percentage of lignified cells, and this seems to indicate that the harshness of fibre is associated with the amount of lignification of fibre cells in the stem.

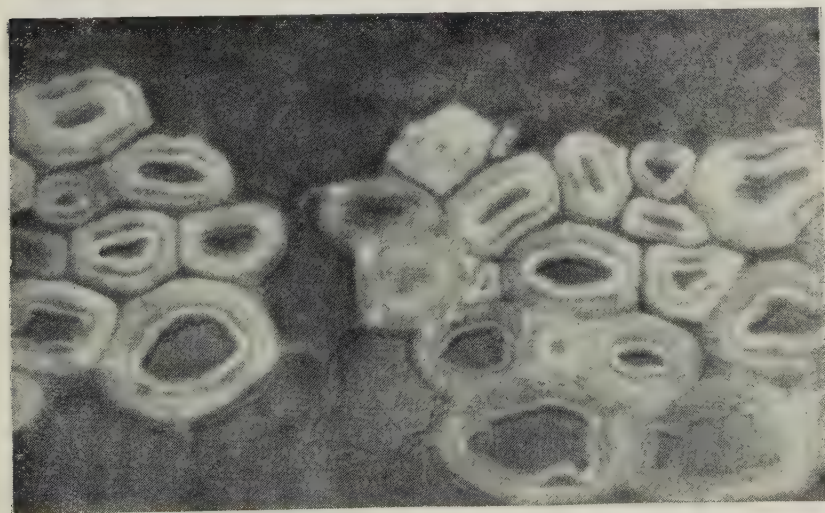


FIG. 3.—Two fibre cells showing the lignified layer around their outer edges, the other fibre cells are thin-walled and show distinct layering. X 1000 (approx.)

The lignified cells are easily recognizable in the unstained transverse section, as the woody layer surrounding the cell is clearly visible, but as a further means of identifying this layer, the sections were stained with aniline sulphate, a temporary stain specific for lignin. Cells showing lignification took up the stain in this layer and appeared bright yellow, as did the cells of the xylem, while all other fibre cells in the section remained unstained. The number of stained cells in each section was counted, and the figure converted to a percentage of the total number of fibre cells in the section. It was found that crops of lower grade contained a higher percentage of lignified cells than those of higher grade. A summary of these results is given in Table V below:—

TABLE V. COMMERCIAL GRADE COMPARED WITH PERCENTAGE OF LIGNIFICATION
(A signifies the lowest grade.)

Grade.	Percentage of Lignified Cells.
A	3.7 - 6.3
B	2.0 - 3.4
C	0.9 - 1.3

There was one exception among the crops analysed, viz crop FL. 27B, graded C, in which the stems contained 3.5 per cent of lignified cells.

Good correlation was also found between grade and the percentage of calculated fibre actually extracted, i.e. the actual fibre yield expressed as a percentage of the calculated total fibre present in the crop. These results are shown in Table VI:—

TABLE VI. CORRELATION OF FIBRE EXTRACTION PERCENTAGE
AND COMMERCIAL GRADE

Crop.	Calculated Fibre Yield.	Actual Fibre Yield.	Percentage Extraction.	Grade.
	(%)	(%)		
MK. 20	19.66	16.84	85	C
FL. 27 C	22.40	13.05	58	C
FL. 27 B	23.28	12.66	54	C
MV. 27 A	18.60	12.94	70	B
FL. 27 A	23.5	11.31	48	B
FL. 8	20.79	10.55	50	B
WT. 50	20.0	8.76	44	B
MV. 28	21.68	7.25	34	A
MV. 40 A	16.2	5.9	36	A
MK. 15 B	17.43	3.50	20	A

These observations emphasize the close relationship between fibre quality and the amount of extractable fibre. It is obvious that one is affected by the other and that neither can be successfully studied alone.

This fact is further illustrated in Table VII below in which a conversion empirical factor is used to estimate the extractable fibre from the calculated fibre percentage and the fibre index, namely,

$$\text{Estimated Fibre Yield} = \frac{\text{Calculated Fibre Percentage}}{\text{Index Number}} \times 5$$

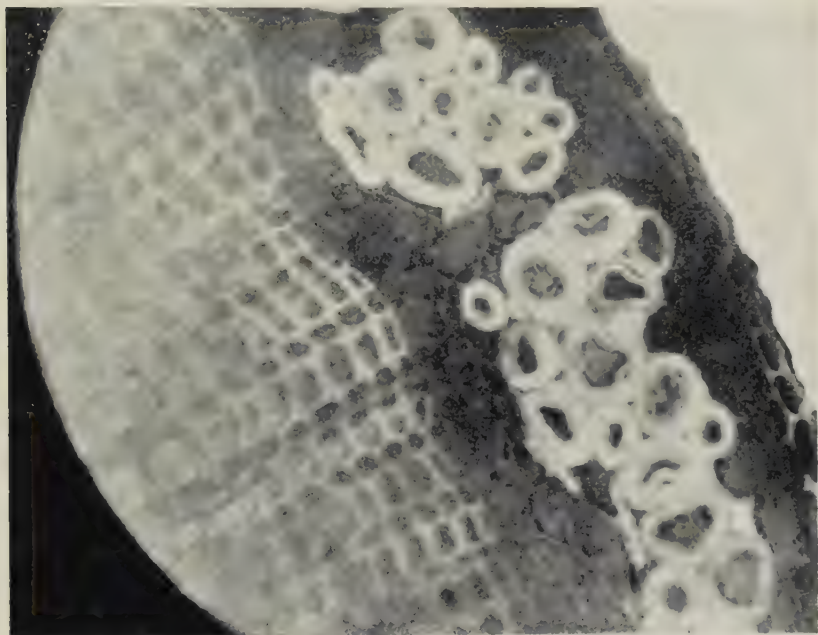


FIG. 4.—Poor fibre cells in transverse section with extreme layering or cleavage. X 500 (approx.)



FIG. 5.—Radial markings on transverse sections of poor fibre cells. X 1400 (approx.)

TABLE VII. COMPARISON BETWEEN ACTUAL AND ESTIMATED FIBRE YIELDS

Crop No.	Estimated Fibre.	Actual Fibre.	Grade.
MK. 20	9.83	16.84	C
FL. 27 C	14.00	13.05	C
FL. 27 B	11.64	12.66	C
MV. 27 A	8.48	12.94	B
FL. 27 A	13.06	11.31	B
FL. 8	9.41	10.55	B
WT. 50	10.00	8.76	B
MV. 28	9.04	7.25	A
MV. 40 A	6.23	5.90	A
MK. 15 B	6.22	3.50	A

Although not included in the fibre index, observations were made on layering and radial markings of fibre cells, in the hope that some light would be thrown on their effect, if any, on quality. Layering is a condition of a cell, in which one or more layers of fibre are clearly distinct, even to the extent of there being an actual separation of the layers. This condition, illustrated in transverse section in Fig. 4, may be supposed to weaken the fibre.

Many fibres show radial markings, as illustrated in Fig. 5, although in all the cells examined these were always confined to the outer layer of the cell wall. The cause of these markings and their effect upon quality is unknown.

The rating used in layering and radial marking is given below, and the results of the analysis in Table VII. In the ten crops examined there appeared to be no association between these characters and the grade of the processed fibre.

LAYERING	RATING
<i>Rating for Layering and Radial Marking</i>	
Present but faint	1
Moderate	2
Strong	3
Actual separation of cell layers or	
Cleavage (a) of one layer	4a
(b) of more than one layer	4b
RADIAL MARKING	
Present (a) on a few fibres	1a
(b) on more than 25 per cent of the fibres	1b
Absent	2

TABLE VIII. LAYERING AND RADIAL MARKING COMPARED WITH FIBRE YIELD

Crop.	Layering.	Radial Marking.	Grade.	Yield. (%)
MK. 20	3.5 b	1 a	C	16.84
FL. 27 C	1	1 a	C	13.05
FL. 27 B	2	1 a	B	12.94
MV. 27 A	1 a	1 a	C	12.66
FL. 27 A	2	1 b	B	11.31
FL. 8	2 b	1 b	B	10.55
WT. 50	2	1 b	B	8.76
MV. 28	1 a	1 a	A	7.25
MV. 40 A	2	1 b	A	5.9
MK. 15 B	2	1 a	A	3.5

Counts were also made of the total number of fibres present in each transverse section, and of the number of fibre bundles per stem. Although these varied significantly between crops, they had no apparent relation to quality.

CONCLUSION AND SUGGESTIONS

This investigation has shown that it may be possible to correlate the microscopic appearance of transverse sections of flax fibres with the grade of the processed fibre, and with the fibre extraction percentage, which is a value calculated from the actual fibre yield and the theoretical total fibre content. The work has also indicated that the amount of lignification in a stem is directly connected with the grade, and appears to be responsible for the harshness associated with poor quality fibre.

The closing of the Research Laboratory at Washdyke prevented further work being carried out along these lines, but the importance of it is stressed before some of the above results may be accepted in their entirety. The difficulty of correlating microscopic grade and commercial grade is great, and it is fully appreciated that the primary characteristics within the stem necessary to produce fibre of high quality can be largely masked by secondary external effects. For example, a relatively harsh flax might have its quality improved by an extension of the time of retting, and another flax, which appeared of high quality when examined microscopically, could be ruined by subsequent exposure of the straw to adverse weather conditions, or to the ravages of a disease such as rust (*Melampsora lini*). Nevertheless, this method of microscopic analysis of stem cross-sections as a means of estimating quality and yield of extractable fibre, offers distinct possibilities for use in the breeding and selection of finer quality flax.

The following suggestions are offered in the hope that they may prove useful lines of investigation for other workers to follow:—

1. To reduce the error due to smallness of sample of a very variable population, breeders might grow on single plant progenies until they have enough material to ret and scutch. A small sample of such material is much more likely to be truly representative of the whole than is a small sample of a commercial crop.
2. The comparison between blue blossom and white blossom linen flax and linseed might help in the interpretation of the significance of the various factors used in compiling the fibre index.
3. The use of manures of various compositions designed to increase or decrease the quality of the fibres.
4. The total amount of fibre in the straw varies much less than the amount of line fibre extracted. Although deterioration between harvest and processing can be serious, it is reasonably certain that inherent fibre quality plays an important part. If variation in quality is reflected in variation of the appearance of the fibre under the microscope, it should be possible to devise a Fibre Index to be used as a factor to convert total fibre (calculated) into extractable fibre.
5. As the six characters included in the Fibre Index may not be of equal importance, investigations are desirable to determine whether or not they should be differentially weighted.

These conclusions, nevertheless, do not preclude the use of this method of microscopic examination of stem cross-sections in the breeding and selection of finer quality flax.

ACKNOWLEDGMENTS

Special acknowledgment is made to Mr. A. O. Gidall, of the Linen Flax Research Laboratory, for devising the method of determining the total fibre content of transverse sections of stems by weighing the tracings of various plant tissues, and for designing and constructing much of the equipment necessary in the making of projections; and to Mr. M. A. Black for his helpful suggestions and advice in the preparation of this paper. Thanks are also due to Mr. I. D. Dick of the Applied Mathematics Laboratory, Department of Scientific and Industrial Research, for analysing the results statistically.

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EFFECT OF CHEMICALS AND ANTIBIOTIC SUBSTANCES ON CROWN-GALL (*Agrobacterium tumefaciens* (Smith and Townsend) Conn.)

I. COLCHICINE AND PENICILLIN

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Summary

Aqueous solutions of the chromosome doubling chemical colchicine, at strengths of 0.5 per cent and 2.0 per cent, tested on marigolds and tomatoes under glass, effectively prevented the formation of bacterial tumours and either caused the death of healthy growing galls or retarded their further development. Colchicine in a concentration of 2.0 per cent was not significantly more effective than in a concentration of 0.5 per cent.

The antibiotic substance, commercial penicillin, at water solution concentrations of 15 and 150 Oxford units per ml., tested on the above hosts, neither prevented gall formation nor delayed the development of growing tumours. There was no significant difference in the results at the two concentrations.

Of the three methods of application, viz. (1) injection; (2) brushing on the solution, and (3) smearing the stem or gall with a lanolin paste containing the solution, the last method was the most effective.

A. tumefaciens was re-isolated from all treatments on marigolds except those of colchicine on growing galls.

INTRODUCTION

Crown-gall (*Agrobacterium tumefaciens* (Smith and Townsend) Conn.) was first recorded in New Zealand by Boucher (1900) on *Prunus persica* Sieb. and Zucc. Cunningham (1925) recorded this disease on *Prunus armeniaca* L., *Prunus avium* L., *Prunus communis* Fritsch, *Prunus domestica* L., *Prunus persica*, *Pyrus communis* L., *Pyrus malus* L., and *Cydonia oblonga* Mill.; Woodhead and Chamberlain (1940) reported it on *Rubus idoeus* L.; and Brien (1942) on *Rubus loganobaccus* Bailey, and *Rheum rhaponticum* L. Recently crown-gall has been observed on *Actinidia chinensis* Planch., *Lycopersicum esculentum* Mill., *Pastinacia sativa* L., and *Rumex obtusifolius* L. While sometimes serious, it is not considered a disease of major economic importance in this country. To date, investigations on control have followed two courses, namely, adjustment of soil pH, and therapeutant treatment. Overseas many chemotherapeutants and antibiotic substances have been tested for their ability to control crown-gall. The alkaloid colchicine has been used with varying success by a number of workers, using different methods of application. Acenaphthene, with similar properties to colchicine (both cause chromosome doubling) has also been used. Many antibiotic substances have been successful *in vitro* in the inhibition of the crown-gall organism, but of these only penicillin appears to have been used *in vivo*. Clove oil, iodine and many other disinfectants have been used experimentally. The present paper deals with two of these treatments, viz. colchicine and penicillin, while others will be dealt with subsequently.

LITERATURE REVIEW

A. Colchicine

Havas (1937) used a 1:10,000 aqueous colchicine solution and reduced the average weight of galls to 0.211 g as compared with 0.444 g in the control plants. Brown (1939) found that 2 per cent colchicine injected into plants after inoculation with *A. tumefaciens* reduced gall size and delayed gall development. A 3 per cent colchicine solution in lanolin, smeared over the inoculated area, delayed the appearance of galls and their ultimate size was only half that of the controls. Brown also found that colchicine solutions brushed on bacterial tumours either inhibited their growth or destroyed them.

B. Penicillin

Waksman *et al.* (1944) in cultural studies found that penicillin containing 470 Oxford units per ml, considerably lowered the viability of *A. tumefaciens*. Brown and Boyle (1945) found that crude penicillin (6 O.U. per ml) injected into soft galls caused their destruction. Tests *in vitro* by the latter authors showed little, if any, difference in sensitivity between *Agrobacterium tumefaciens* and *Staphylococcus aureus*.

MATERIALS AND METHODS

Colchicine and penicillin were applied to crown-gall inoculated plants, either at time of inoculation or after development of galls. Two concentrations of materials with three methods of application were used.

French Marigold (*Tagetes patula* L.) and tomato (*Lycopersicum esculentum* Mill.) were used as the host plants. The plants were grown under glass in 4 in pots of sterilized compost soil mixture. Throughout the experiment air temperatures were maintained at 60° to 80°F. When one month old, the plants were divided into two equal groups and one group was inoculated with the crown-gall organism. Thirty-three days later, the second group was inoculated and plants in both groups were treated. By this arrangement all plants were the same age and all treatments were applied on the same day. Each treatment was applied on six plants of each host. Beef-peptone broth cultures from a previously tested active strain of *A. tumefaciens* were used for the inoculations, 0.5 ml being injected into each plant with a Cornwall pipetting syringe. The needle was forced in most plants, right through the stem midway between the cotyledonary leaves and the first true leaves, so that the stem was usually punctured at two points.

Treatments

Materials were used at the following strengths:—

- (1) Amorphous colchicine 0.5 per cent aqueous solution (Colchicine 1).
- (2) Amorphous colchicine 2.0 per cent aqueous solution (Colchicine 2).
- (3) Commercial penicillin in water, which assayed 15 Oxford units per ml* (Penicillin 1).
- (4) Commercial penicillin in water 150 O.U. per ml* (Penicillin 2).
- (5) Water (control).

*The penicillin was supplied and assayed by Dr T. R. Vernon, Plant Chemistry Laboratory, Department of Scientific and Industrial Research, Auckland.

Three methods of application were used.

- A. By injecting the solution into the plant or gall.
- B. By smearing the inoculated area or gall with a lanolin paste containing the solution.
- C. By brushing the solution on to the inoculated stem or gall.

TABLE I. EXPERIMENT 1. THE EFFECT OF PENICILLIN AND COLCHICINE ON
DEVELOPMENT OF CROWN-GALL FOLLOWING TREATMENT AT TIME OF INOCULATION

Host Plant	Treatment		No. of inoculated points	Galls formed		Average increase in diameter of galls	
	Material	Method of application		No.	(%) *	Per gall (cm) **	For each treatment (cm)
Marigold	Penicillin 1 15 O.U.	Injected	9	9	100	1.11	} 0.93
		Smearcd	10	7	70	0.49	
		Brushed	10	9	90	1.19	
	Penicillin 2 150 O.U.	Injected	11	9	82	1.03	} 0.89
		Smearcd	10	5	50	0.41	
		Brushed	6	6	100	1.24	
	Colchicine 1 0.5%	Injected	12	0	0	0	} 0
		Smearcd	12	0	0	0	
		Brushed	11	0	0	0	
	Colchicine 2 2.0%	Injected	10	0	0	0	} 0
		Smearcd	12	0	0	0	
		Brushed	11	0	0	0	
	Control Water	Injected	11	10	91	1.21	} 0.99
		Smearcd	12	10	83	0.72	
		Brushed	9	9	100	1.03	
Tomato	Penicillin 1 15 O.U.	Injected	12	10	83	1.09	} 0.73
		Smearcd	12	9	75	0.47	
		Brushed	11	8	73	0.62	
	Penicillin 2 150 O.U.	Injected	12	9	75	0.76	} 1.05
		Smearcd	12	12	100	1.15	
		Brushed	12	12	100	1.24	
	Colchicine 1 0.5%	Injected	12	7	58	0.19	} 0.26
		Smearcd	12	5	42	0.36	
		Brushed	12	4	33	0.22	
	Colchicine 2 2.0%	Injected	12	2	17	0.15	} 0.12
		Smearcd	12	2	17	0.07	
		Brushed	12	3	25	0.14	
	Control Water	Injected	12	9	75	0.75	} 0.75
		Smearcd	12	12	100	0.67	
		Brushed	12	8	67	0.83	

* Difference for significance at 5%: 1. Marigold 52.0
2. Tomato 43.0

** A. For Marigold.

Average increase in horizontal diameter of galls in cm 72 days after treatment. Difference for significance between these totals at 5% = 0.32.

B. For Tomato.

Average increase in cross section area of galls in sq. cm 75 days after treatment. Difference for significance at 5% = 0.69 and at 1% = 0.93.

Experiment 1. Treatment at Time of Inoculation

A. Immediately after inoculation the plants were injected with 0.5 ml of one of the above solutions 0.5 cm below the point of inoculation. Usually the plant was not able to retain all of the solution that was injected.

B. An area of approximately 3 sq. cm of the stem, around each point of inoculation was thickly smeared with a lanolin paste containing one of the above solutions.**

C. As soon as excess inoculum had drained away, an area of approximately 3 sq. cm surrounding the inoculated area was brushed with one of the solutions.

Experiment 2. Treatment of Growing Galls

Only vigorously growing galls formed by inoculation 33 days previously, were used. On the majority of plants two galls were formed, one each at point of entry and exit of the inoculating needle. Additional galls were sometimes formed at other points on plants, but treatments were applied only to the galls formed at points of inoculation. Concentration of materials, and methods of treatment by smearing and brushing were the same as for Experiment 1. Application by injection, in this instance, involved making several needle punctures into each gall in order that the bulk of the 0.5 ml of solution would be retained by the gall.

TABLE II. STATISTICAL ANALYSIS OF RESULTS IN TABLE I

Host	Comparison	Difference	Difference for significance at		Result
			5%	1%	
Marigold	Penicillin 1 V Water	-0.06	0.19	0.25	Highly significant
	Penicillin 2 V Water	-0.10			
	Penicillin 1 V Penicillin 2	0.04			
	Colchicine 1 V Water	-0.99			
	Colchicine 2 V Water	-0.99	0.56	0.76	Highly significant
	Colchicine 1 V Colchicine 2	0			
	Methods. Injected V Smeared	1.74			
	Injected V Brushed	-0.11			
	Brushed V Smeared	1.85			
Tomato	Penicillin 1 V Water	-0.02	0.40	0.54	Significant
	Penicillin 2 V Water	0.30			
	Penicillin 1 V Penicillin 2	-0.32			
	Colchicine 1 V Water	-0.49			
	Colchicine 2 V Water	-0.63	1.55		Highly significant
	Colchicine 1 V Colchicine 2	0.14			
	Methods. Injected V Smeared	0.22			
	Injected V Brushed	-0.11			
	Brushed V Smeared	0.33			

** Preparation of lanolin paste mixture.

60 ml of melted crude lanolin was mixed with 1 ml soyabean oil and divided into 10 ml portions, cooled, then each portion was beaten to a stiff white paste. To each 10 ml portion of this paste 10 ml of the required solution was slowly added, 2 ml at a time. Only by mixing in this manner was it possible to incorporate all of the solution into the paste.

Recording Results

The horizontal diameter of all galls on marigolds was measured with sliding vernier calipers at intervals of 5-7 days, beginning 28 days after inoculation and continuing for a further 44 days. Because of the asymmetrical shape of the galls on tomato it was necessary to make horizontal and vertical measurements, which gave approximate cross section area. These were taken three times in the above period. Colour changes and other differences were noted.

**TABLE III. EXPERIMENT 2. THE EFFECT OF TREATMENT WITH
PENICILLIN AND COLCHICINE ON 33-DAY-OLD CROWN-GALLS**

Host	Treatment		No. of galls treated	Galls killed by treatment	Average increase in diameter of galls	
	Material	Method of application			Per galls (cm)	For each treatment (cm)
Marigold	Penicillin 1 15 O.U.	Injected	11	0	0.82	} 0.97
		Smearcd	12	0	1.16	
		Brushed	12	0	0.94	
	Penicillin 2 150 O.U.	Injected	11	0	1.41	} 1.33
		Smearcd	12	0	1.24	
		Brushed	12	0	1.34	
	Colchicine 0.5%	Injected	12	11	0.12	} 0.21
		Smearcd	12	8	0.40	
		Brushed	12	11	0.10	
	Colchicine 2.0%	Injected	12	10	0.22	} 0.12
		Smearcd	12	12	0.07	
		Brushed	12	11	0.08	
	Control Water	Injected	12	0	1.11	} 1.18
		Smearcd	11	0	0.97	
		Brushed	12	0	1.45	
Tomato					Average increase in cross section area of galls	
					(sq.cm)	(sq.cm)
	Penicillin 1 15 O.U.	Injected	12	0	0.69	} 0.33
		Smearcd	12	0	-0.01	
		Brushed	12	0	0.30	
	Penicillin 2 150 O.U.	Injected	12	0	1.00	} 0.67
		Smearcd	11	0	0.40	
		Brushed	12	0	0.62	
	Colchicine 1 0.5%	Injected	12	0	0.15	} 0.14
		Smearcd	12	0	0.07	
		Brushed	12	0	0.19	
	Colchicine 2 2.0%	Injected	12	0	0.03	} 0.03
		Smearcd	12	0	-0.10	
		Brushed	12	0	0.16	
	Control Water	Injected	12	0	0.74	} 0.43
		Smearcd	12	0	-0.09	
		Brushed	11	0	0.64	

* For Marigold. Average increase in horizontal diameter of galls in cm 72 days after treatment. Difference for significance between these totals at 5% = 0.46 and at 1% = 0.61.

For Tomato. Average increase in cross section area of galls in sq. cm. 75 days after treatment. Difference for significance at 5% = 0.64 and at 1% = 0.89.

Reisolation

One hundred and seventeen days after treatment an attempt was made to reisolate the pathogen from marigold plants in all treatments. Where gall development was inhibited, isolations were made from the stems of plants at the points of inoculation. All bacteria isolated were then re-inoculated into the stems of young French Marigolds.

RESULTS

The results of Experiment 1 are contained in Table I and those of Experiment 2 in Table III. Statistical analyses of these results are shown in Tables II and IV respectively.

Experiment 1. Treatment at Time of Inoculation

Colchicine.—On marigold both concentrations of colchicine prevented gall formation within the area covered by the colchicine, but did not prevent the development of galls on other parts of several plants. On tomatoes treated with 0.5 per cent colchicine, 16 galls developed out of a possible 36, and on plants treated with the 2.0 per cent solution seven galls were formed within the 36 treated zones. The average size of all galls in these colchicine treatments was less than in other treatments.

On both marigold and tomato, the differences between colchicine and water, in the number of galls formed and in gall size were significant.

Penicillin.—Results of penicillin treatments on marigolds and tomatoes were not significantly different from the water treated controls. The cause of the increased gall growth on tomato by treatment with Penicillin 2, either smeared or brushed on the plant stems, is not known.

Methods of Application.—Since no galls formed on parts of stems of marigolds treated with colchicine, a comparison of methods of application is not possible; from the penicillin and water treatments, however,

TABLE IV. STATISTICAL ANALYSIS OF RESULTS IN TABLE III

Host	Comparison	Difference	Difference for significance at		Result
			5%	1%	
Marigold	Penicillin 1 V Water	-0.21	0.27	0.36	.
	Penicillin 2 V Water	0.15			.
	Penicillin 1 V Penicillin 2	-0.36			Highly significant
	Colchicine 1 V Water	-0.97			"
	Colchicine 2 V Water	-1.05			.
	Colchicine 1 V Colchicine 2	0.08			.
	Methods. Injected V Smeared	-0.16	1.04		.
	Injected V Brushed	-0.23			.
	Brushed V Smeared	0.07			.
Tomato	Penicillin 1 V Water	-0.10	0.37	0.51	.
	Penicillin 2 V Water	0.24			.
	Penicillin 1 V Penicillin 2	-0.35			.
	Colchicine 1 V Water	-0.29			.
	Colchicine 2 V Water	-0.40			Significant
	Colchicine 1 V Colchicine 2	0.11			.
	Methods. Injected V Smeared	2.34	1.43	1.98	Highly significant
	Injected V Brushed	0.70			.
	Brushed V Smeared	1.64			Significant

it can be seen that the lanolin paste application was significantly better than the other methods, between which there was no significant difference. On tomatoes the three methods of application of either colchicine or penicillin showed no significant differences.

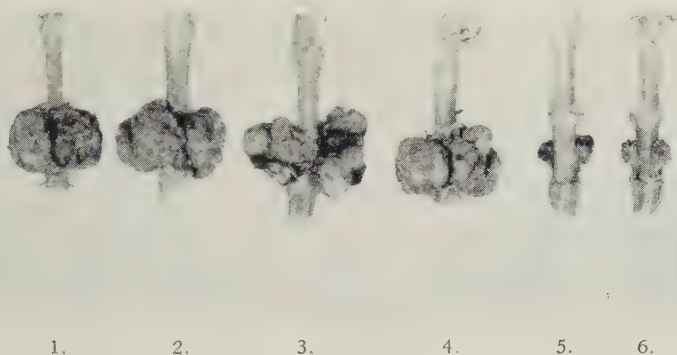


FIG. 1.—*The Effects of Colchicine and Penicillin on Crown-galls.*

A comparison of gall sizes on marigold stems (cut off at ground level) 72 days after treatment.

1. Control. Water applied in a lanolin paste.
2. Control. Water brushed on.
3. Penicillin 2. (150 O.U.) smeared on in a lanolin paste.
4. Penicillin 2 brushed on.
5. Colchicine 2 (2.0 per cent) smeared on in lanolin paste.
6. Colchicine 2 brushed on.

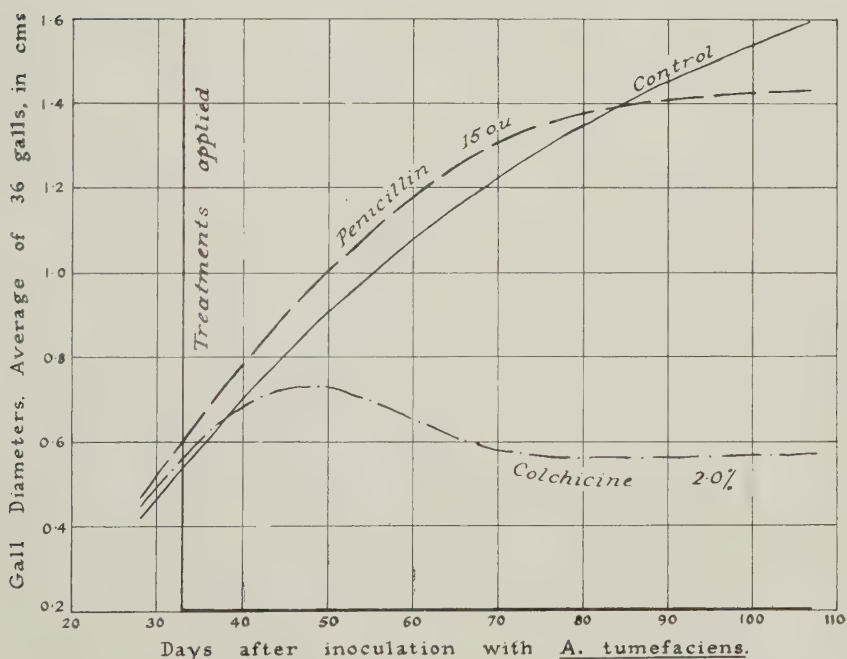


FIG. 2.—*The Effect of Treatment with Penicillin and Colchicine on 33-day-old galls on Marigold.*

Experiment 2. Treatment of Growing Galls

Colchicine.—The results show that colchicine at each strength on both hosts effectively killed galls or prevented their further growth. The difference between the two concentrations was not significant. On marigolds the retarding effect of colchicine on gall size was observed within seven days after treatment (Fig. 2), and after 14 days these galls had reached their maximum size. Thereafter, treatment caused galls to wither gradually until after 45 days the majority was dead. The slight rise shown in the graph for colchicine from 90 days to 110 days after inoculation, was due to one gall which continued to grow, whereas all other galls were either withered and dead, or had ceased to grow.

Twelve days after the treatments were applied on marigolds, most colchicine treated galls showed definite blackening which steadily became more pronounced as they withered. Some atrophied and dropped off.

Penicillin.—Penicillin on both marigolds and tomatoes did not retard the growth of galls. On marigolds slight blackening of the gall epidermis appeared after 12 days, but these galls continued to grow, and the blackened appearance disappeared after a further 10 to 15 days. The highly significant difference between Penicillin 1 and Penicillin 2 on marigolds was due to the latter concentration increasing gall growth. The reason for this is not known.

Methods of Application.—On marigold, no one method was significantly different from any other. On tomato, application of the solution in a lanolin paste was significantly better than either injection or brushing.

Results of Re-isolation

Cultures from Experiment 1.—Positive crown-gall cultures were isolated from plants in all treatments, including those plants on which galls were prevented from forming. The organism was also re-isolated from several galls which had formed on plants at points other than those of inoculation.

Cultures from Experiment 2.—*A. tumefaciens* was isolated from penicillin and water treatments, but could not be isolated from any of the colchicine treated galls.

CONCLUSIONS

A 0.5 per cent aqueous solution of colchicine tested on marigold and tomato proved to be almost as effective as 2.0 per cent solution in preventing the formation of bacterial galls and in causing either the death of growing tumours or retarding their further development. The results suggest that with an improved technique of application, even weaker concentrations may give similar control.

Commercial penicillin solutions at concentrations of 15 and 150 Oxford units per ml did not prevent gall formation nor did they kill or retard the development of growing tumours. In some instances penicillin actually increased the vigour of galls. The fact that a refined preparation was used, whereas Brown and Boyle (1945) used crude penicillin may be the reason for this complete lack of success.

On marigolds, at time of inoculation, smearing was significantly better than either injection or brushing, while on tomatoes no one method was superior to the others. In treatment of growing galls, there were no significant differences between methods on marigold, but on tomato, smearing was the most effective. It appears from the control treatments

that lanolin alone had a retarding effect on gall growth and that the action of some treatments was masked by this effect. The reduced gall size in some of the penicillin treatments applied with lanolin may be due solely to this latter material. On the other hand, the comparatively increased growth of marigold galls treated with 0.5 per cent colchicine in lanolin could have been caused by the lanolin diluting the colchicine strength and making it too slowly available.

The crown-gall organism was re-isolated from all treatments on marigolds except those of colchicine on growing galls. Since the pathogen was re-isolated from stems of plants on which colchicine prevents gall development, it is considered that this chemical merely exerts a bacteriostatic action on *A. tumefaciens*. The advanced state of decomposition was probably the reason for the failure to recover the organism from the colchicine-treated galls.

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TRIALS OF SOME COMMERCIAL PLANT-GROWTH SUBSTANCES FOR THE CONTROL OF FRUIT-DROP

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Summary

Trials of four commercial preparations of synthetic growth substances for reducing pre-harvest drop in apples showed:—

(1) Fruit Fix was the most efficient material, but owing to its phytocidal properties was a potential danger to the growth of apple trees and other crops.

(2) Clingspray, Estone, and Fruitone, also gave good commercial control of fruit-drop, Clingspray being the most and Fruitone the least effective.

(3) All preparations became effective in two to four days.

(4) Maximum control was achieved six to eight days after application.

(5) Fruit Fix controlled fruit-drop for eight weeks' duration of control in other treatments ranging from ten days to three weeks according to preparation and variety of apple.

(6) In these trials an interval of two weeks between application and harvesting date proved satisfactory. Fruit Fix, because of its lasting effect, could be applied earlier than this.

(7) Fruit-drop was more difficult to control on Jonathan than on Parlin's Beauty trees.

INTRODUCTION

IN some seasons, particularly in exposed localities, a number of apple varieties tend to shed a portion of their crop shortly before it is ready to be picked. The prevention of this "pre-harvest drop" would entail considerable financial gain to growers, especially among those early varieties where heavy drops occur. Since the discovery by Gardner, Marth and Batjer (1939) that dilute sprays of certain "growth substances" give considerable control of fruit-drop, wide use of commercial preparations of these substances has been reported in the United States of America, England, and Australia. In order to test commercial synthetic growth substances under New Zealand conditions, two trials were carried out on apple trees at the Plant Diseases Division, Mt. Albert, Auckland.

TRIAL I

Material and Methods

Six-year-old Parlin's Beauty trees on E.M. No. IX dwarf stocks were used for this trial. An area containing forty trees was divided into randomized blocks, giving eight replications of the following five treatments:—

(i) *Control*—tap-water only;

(ii) *Estone*—a preparation containing α -naphthalene acetic acid (14.8 ml per 4 gal)

(iii) *Clingspray*—a preparation containing α -naphthalene acetic acid (4.5 ml per 4 gal)

(iv) *Fruitone*—a preparation described as a “multi-hormone spray” including α -naphthalene acetic acid and α -naphthyl acetamide (9 g per 4 gal)

(v) *Fruit Fix*—a preparation described as a “mixture of plant hormones”. (9 g per 4 gal)

On 15 March 1948, approximately two weeks before the normal picking date, fruit began to drop steadily. Materials were prepared according to the manufacturer's instructions and sprayed on the trees, using a bucket pump at the rate of approximately one and a half gallons per bushel of fruit. The weather was warm, humid, overcast, and calm. To provide data on the degree and duration of control by the different treatments, windfalls from each tree were collected and counted daily, until all fruit had dropped from controls. Fruit-drop was heaviest during periods of high wind on 20-23 March, 27 March, 1 April, and 5-7 April. After the normal picking period, fruit began to ripen and towards the end of the experiment many fruits cracked, rotted, and dropped, leaving the stalks on the tree. These stalkless fruits, together with any that were birdpecked or codling-moth infested were not included in either the daily count of windfalls or the total crop. Fruits which dropped prior to 15 March were also excluded from crop totals.

Results

Table I shows the amount of fruit retained on control and treated trees on successive dates after spraying. Two weeks after spraying, when fruit would normally have been picked, all treatments gave satisfactory protection and reduced loss of fruit from 34 per cent to approximately 10-16 per cent of total crop. At that time there were no significant differences between treatments. However, at later dates, differences in efficiency as indicated by saving of crop, became marked. After three weeks, Fruit Fix was significantly more effective than any other treatment with a maximum saving in the eighth week of 64 per cent of total crop. Fruit Fix still maintained a significant saving at the end of the experimental period of ten weeks. Although Clingspray was almost as efficient as Fruit Fix for the first four weeks, maximum saving was only 39 per cent of total crop in the fifth week and there was no significant difference from controls by the seventh week. Estone reached a maximum saving of 31 per cent of total crop during the fourth week. During the fifth week, Estone was significantly less effective than either Fruit Fix or Clingspray and significant saving ceased during the seventh week. Fruitone reached a maximum saving of 27 per cent in the third week and there was no significant difference from controls after the fifth week.

While this analysis based on cumulative windfall totals shows the relative effectiveness of the sprays, it does not give a correct indication of the periods during which actual control of fruit-drop was achieved. Therefore another analysis of fruit-drop was made in two-day periods, covering the first four weeks after treatment, to determine the following details :—

- (1) interval between application of treatment and control of drop;
- (2) period of maximum control;
- (3) time of cessation of control.

TABLE I. EFFECT OF FOUR COMMERCIAL GROWTH SUBSTANCES ON FRUIT-DROP FROM PARLIN'S BEAUTY APPLE TREES: MEAN CROP RETAINED (As Percentage of Total Crop).

Weeks After Treatment	1	2	3	4	5	6	7	8	9	10
Control	81.5	66.0	50.3	37.0	28.3	17.6	9.2	2.3	0.0	0.0
Fruit Fix	93.0*	90.2*	88.9*	87.0*	79.3*	76.5*	70.5*	66.5*	50.5*	40.2*
Clingspray	93.9	90.1*	82.6*	75.4*	67.5*	40.3*	27.2	16.6	6.5	2.0
Estone	94.1*	84.8*	77.8*	68.5*	54.3*	37.4*	28.4	13.3	7.8	1.4
Fruitone	93.5*	85.6*	77.0*	61.9*	46.8*	26.0	19.4	7.6	3.0	0.4
Difference for Significance at 5%	5.74	9.02	9.83	11.48	11.19	11.15	38.54	17.58	9.80	4.84

* Indicates significant difference between treatment and control.

TABLE II. EFFECT OF FOUR COMMERCIAL GROWTH SUBSTANCES ON FRUIT-DROP FROM PARLIN'S BEAUTY APPLE TREES: MEAN FRUIT-DROP (As Percentage of Total Crop)

Mean Drop (% Total Crop)	Actual Means	Days After Treatment															
		2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32
Control	2.2	2.4	6.1	7.2	1.6	6.2	6.3	1.3	3.3	1.3	5.4	5.4	7.5	6.5	2.5	2.6	3.6
Fruit Fix	2.2	0.2*	5.7	1.8*	0.0	0.8*	2.1*	0.0*	0.1	0.0*	1.1	0.1*	0.1*	1.1*	1.1	0.1	1.6
Clingspray	1.1	0.1*	5.6	0.6*	1.1	0.6*	2.8*	1.9	1.9	1.1	1.1	2.1	4.2	2.2	2.2	2.2	6.3
Estone	1.1*	1.2	3.5*	0.5*	1.1	3.9	4.1*	2.1	2.1	2.1	1.2	3.2	3.2	2.3	3.3	2.3	5.4
Fruitone	0.0*	0.1	3.4*	2.6*	0.8	2.8*	3.1*	0.1	3.1	1.1	1.1	5.2	3.2	3.2	6.3	3.3	4.4
Control	6.8	6.5	14.1	15.4	2.3	11.8	15.6	2.8	7.0	4.2	12.2	13.6	13.6	11.5	8.4	5.8	7.9
Fruit Fix	3.9	1.0	10.1	1.4	0.0	1.0	8.1	1.0	1.0	0.0	1.8	2.0	5.5	2.8	4.7	0.0	6.3
Clingspray	3.0	0.0	10.8	1.0	1.7	1.2	5.8	1.7	4.3	4.3	3.3	6.6	8.6	4.8	6.2	5.7	9.5
Estone	2.0	4.3	9.3	0.8	1.6	8.8	8.7	4.9	6.2	1.6	8.1	8.3	8.3	6.6	7.3	5.3	9.7
Fruitone	1.2	3.6	7.1	5.1	1.0	3.2	7.7	1.0	5.0	2.9	8.5	7.7	7.7	7.9	9.9	8.2	11.6
Difference for Significance at 5%	4.24	4.12	5.66	4.30	4.71	6.35	6.79	4.26	6.00	4.79	6.31	6.10	6.10	6.87	6.59	6.14	7.29

* Indicates significant difference between treatment and control.

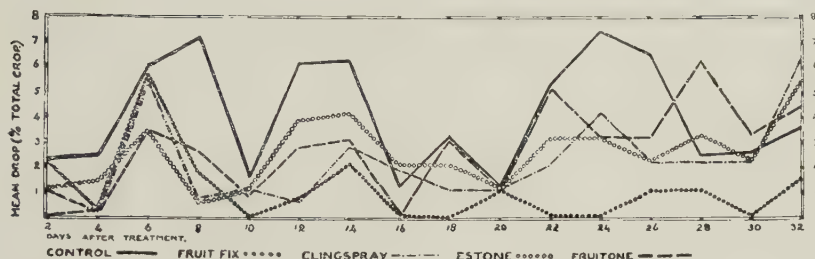


FIG. 1.—Effect of four commercial growth substances on fruit-drop from Parlin's Beauty apple trees. Mean fruit-drop (as percentage of total crop).

These results are tabulated in Table II and represented graphically in Fig. 1. The rate of fall of controls as indicated in Fig. 1, shows that during high wind as much as 6-8 per cent of total crop was lost in a two-day period, while in a similar period of calm weather only 2-3 per cent was lost.

Fruitone and Estone began to control fruit-drop one to two days after date of application, but Clingspray and Fruit Fix did not become effective before the third or fourth day. All sprays reached maximum control during periods of strong winds six to eight days after application. Thereafter, the effect of all sprays except Fruit Fix gradually decreased until treated trees began to drop fruit at a faster rate than controls. In trees treated with Fruitone and Estone, this occurred 28 days after spraying and in Clingspray treated trees, 32 days after spraying. As the significance of control by Fruit Fix was obvious throughout the trial, results were not analysed statistically after 32 days. Fruit Fix steadily maintained a rate of approximately 1-2 per cent fruit-drop per two-day period until the eighth week when control of fruit-drop decreased.

There was no evidence of injury to foliage by any treatment during the experimental period, but new growth produced by Fruit-Fix-treated trees in the following season showed malformation typical of the injury induced by weed-killing hormones (Atkinson 1947).

TRIAL II

Material and Methods

Nine-year-old Jonathan trees on Northern Spy rootstock were used for this trial. An area of twelve trees was divided into randomized blocks giving four replications of the following three treatments:—

- (i) *Control*—tap water only;
- (ii) *Estone*;
- (iii) *Clingspray*.

To obtain information on the rate of fruit-drop over the whole experimental block, windfalls were collected and counted daily for a week prior to treatment. On 26 March 1948, two weeks before normal picking date, fruit began to drop steadily, and treatments were applied using a bucket pump. The weather was warm and overcast with a light breeze. After spraying, windfalls were recorded daily. Fruit-drop was heaviest during periods of high wind on 27 March, 1 April, 7 April, 16 April,

and 19 April. Birdpecked, stalkless, and codling-moth infested fruits, were not included in either daily counts of windfalls or in total crop. On 23 April, two weeks after the normal date of harvesting, fruit remaining on trees was picked and examined for colour, maturity, and incidence of Jonathan spot.

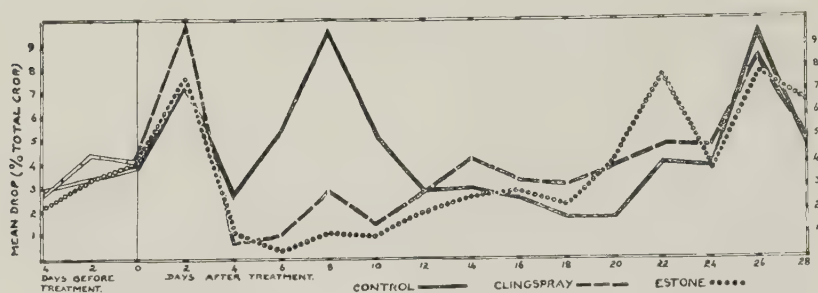


FIG. 2.—Effect of two commercial growth substances on fruit-drop from Jonathan apple trees. Mean fruit-drop (as percentage of total crop).

Results

Over the six-day period prior to spraying, there was no significant difference in mean rate of fruit-drop between treatment blocks. Table III shows the amount of fruit retained on control and treated trees on successive dates after spraying. It may be seen that both treatments achieved maximum saving of crop two weeks after spraying when fruit would normally have been picked. At this stage, Clingspray was significantly more effective than Estone, i.e. Clingspray reduced the loss of fruit from 46 per cent to 24 per cent while Estone reduced it to 33 per cent of total crop. As in the first trial, an analysis in two-day periods was made, the results being tabulated in Table IV. The rate of fall in control trees (Fig. 2) shows that in periods of high winds as much as 7-10 per cent of total crop was lost in a two-day period, while in a similar period of calm only 2-3 per cent was lost. Both sprays began to control fruit-drop in three to four days and reached maximum control eight to ten days after treatment. Thereafter the effect of sprays gradually diminished until treated trees began to drop fruit at a faster rate than controls. With Estone this increased rate of dropping occurred 12 to 14 days after treatment and with Clingspray 14 to 16 days after treatment.

Examination of tree-ripened fruit, picked a month after treatment, showed that Clingspray had no effect on maturity of fruit or incidence of Jonathan spot. Estone had a small but significant effect in hastening maturity of fruit. This was indicated by an increase in percentage of coloured fruit, intensity of colour, sweetness, and storage breakdown. Incidence of Jonathan-spot was reduced on fruit treated with Estone.

DISCUSSION

Results show that application of commercial preparations of growth substances gave significant control of pre-harvest fruit-drop. On Parlin's Beauty, at the time when fruit would normally have been picked, all treatments were equally effective, but at a similar stage on Jonathans, Clingspray was more effective than Estone.

TABLE III. EFFECT OF TWO COMMERCIAL GROWTH SUBSTANCES ON FRUIT-DROP FROM JONATHAN APPLE TREES: MEAN CROP RETAINED (As Percentage Total Crop).

Mean Crop Retained (% Total Crop)	Pre-Treatment Period	Weeks After Treatment			
		1	2	3	4
Control	92.4	74.0	53.9	47.5	25.6
Clingspray	92.6	81.5**	75.8**	66.0**	40.6*
Estone	90.4	77.2*	66.8*	55.7*	32.8
Difference for Significance at 5%	3.05	3.17	4.80	3.27	8.22

* Indicates a significant difference between treatment and control.

** Indicates significant difference between treatments also.

TABLE IV. EFFECT OF TWO COMMERCIAL GROWTH SUBSTANCES ON FRUIT-DROP FROM JONATHAN APPLE TREES: MEAN FRUIT-DROP (As Percentage Total Crop)

Mean Drop (% Total Crop)	Days Before Treatment				Days After Treatment															
	6	4	2	0	2	4	6	8	10	12	14	16	18	20	22	24	26	28		
Control	2.9	3.3	3.8	7.5	2.7	5.3	9.5	5.1	2.8	2.9	2.5	1.7	1.7	1.7	4.0	3.9	9.5	4.8		
Clingspray	2.2	3.3	3.9	7.7	1.0*	0.3*	1.0*	0.9*	1.9	2.5	2.8	2.2	2.2	4.2*	7.7	3.8	7.7	6.5		
Estone	2.8	4.4	4.2	9.0	0.7*	1.0*	2.7*	1.4*	2.7	4.2	3.3	3.1*	3.1*	3.9*	4.8	4.7	8.5	5.0		
Control	9.8	10.1	11.2	15.8	9.0	12.9	17.8	12.6	8.3	9.6	8.9	7.5	7.5	7.3	11.4	11.0	17.3	12.0		
Clingspray	8.6	10.2	11.3	16.1	5.4	2.4	4.6	5.4	7.7	8.8	9.1	8.1	8.1	11.6	15.6	10.9	16.1	14.7		
Estone	8.9	11.9	11.7	18.1	4.7	5.8	9.3	6.4	8.9	11.8	10.3	10.1	10.1	11.4	12.4	12.3	16.8	12.8		
Difference for Significance at 5%	3.8	2.3	2.5	2.9	3.4	4.0	5.3	5.5	5.0	3.9	4.5	1.9	1.9	2.6	4.7	3.1	6.4	3.4		

From the two trials, it appeared that all treatments required from two to four days to produce a significant reduction in fruit-drop, and reached maximum control in six to eight days. The rate at which control diminished after this date appeared to depend on the preparation and the variety of apple. For instance, on Parlin's Beauty the preparations reduced fruit-drop for approximately four weeks, but on Jonathans fruit-drop proved more difficult to control, the effect lasting for only two weeks. Fruit Fix was the most efficient preparation, maintaining a more effective control for a longer period than any other treatment. All three preparations known to contain α -naphthalene acetic acid produced satisfactory control, but Clingspray was significantly more effective than Estone, and Estone was slightly more effective than Fruitone.

To achieve maximum saving of crop, preparations should be applied at such a time that period of maximum control coincides with period of maximum fall. It was evident that in these trials maximum saving was achieved, i.e. maximum control and maximum fall did coincide. None of the preparations had any phytocidal action on the trees during the experimental period, but Fruit Fix produced malformed foliage in the next season. A slight increase in rate of ripening of treated trees was noted in fruit treated with Estone.

Overseas experiments (Batjer and Thompson 1946) have shown that the application of sprays containing hormones of the weed-killing group to control fruit-drop on apple trees, produced lasting effects similar to those of Fruit Fix. To find the effect of Fruit Fix on tomatoes, which are highly susceptible to injury by weed-killing hormones, twelve plants were sprayed at the strength used in the fruit-drop experiment, i.e. according to the manufacturer's instructions. Subsequent growth on every plant showed symptoms of injury that follow from the application of hormone weedkillers containing 2, 4-D (Atkinson 1947). Hence, in spite of its excellence as a spray for reducing fruit-drop, Fruit Fix, because of its phytocidal activity, is a potential danger to apple trees and to other crops.

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SCION ROOTING IN GRAVENSTEIN NURSE GRAFTS

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Summary

Irrespective of rootstock a period of two years was required to secure a high percentage of rooted scions in Gravenstein nurse grafts on Northern Spy and East Malling No. IX rootstocks. Observations on limited material indicated that scion rooting was more frequent in grafts on the vigorous Northern Spy than on the dwarfing No. IX. Inversion of piece root increased the percentage of rooted scions on No. IX, but the increase was largely offset by a high mortality.

Wired grafts excepted, scion roots in almost all grafts were confined to the region of graft union and removal of nurse roots would have been difficult. Comparatively weak roots were formed clear of the union when scions were constricted by wiring at the end of the first year.

INTRODUCTION

Observations recorded here were made on a small number of nurse grafts, consisting of scions of Gravenstein apple on vegetatively raised Northern Spy and East Malling No. IX rootstocks. Included in grafts on No. IX were a number in which the piece roots were inverted, following the method described by Kerr (1935). To permit deep planting of grafts and consequent encouragement of scion rooting, 10-inch scions were whip grafted on 3-inch piece roots. Grafts were planted in the open in deep loam soil, leaving not more than two buds of the scion above ground.

A year after planting the grafts were lifted and examined for scion rooting. Before replanting, a single loop of 28 gauge copper wire was twisted firmly round a few of the grafts on Northern Spy stock, at approximately 1 inch above the union. All grafts were then replanted and left undisturbed for a further year, when a final examination was made.

INCIDENCE OF SCION ROOTING

Details of grafts planted, survival and scion rooting are given in Table I. Considered as a whole, the results show that a period of two years was necessary to obtain a high percentage of scion-rooted grafts. While the material under observation was insufficient for valid comparison of effect of rootstock or inversion of piece root, certain trends are nevertheless evident. Scion rooting occurred more frequently in grafts on the vigorous Northern Spy than on the dwarfing No. IX stock. Inversion of piece root increased the percentage of rooted scions on No. IX stock, but the increase was almost wholly offset by the mortality in scions grafted by this method.

Wiring of grafts at the end of the first year induced 66.7 per cent of the scions to form roots above the constriction.

Wherever scion roots developed in the first year, they were small, and nurse roots could not have been removed with safety at this stage.

TABLE I. INCIDENCE OF SCION ROOTING IN GRAVENSTEIN NURSE GRAFTS

Rootstock.	No. planted Oct. 1946.	Alive Sept. 1947.		Scion rooted.		No. replanted Sept. 1947.		Alive Sept. 1948.		Scion rooted.	
		No.	Per cent.	No.	Per cent.			No.	Per cent.	No.	Per cent.
Northern Spy	44	16	36.4	3	18.8			5*	100.0	3	60.0
" (wired)	—	—	—	—	—			10	90.0	6	66.7†
Malling No. 1X	15	12	80.0	1	8.3			12	91.7	2	18.2
" (inverted piece root)	15	9	60.0	1	11.1			9	44.4	2	50.0

* Did not include scion-rooted grafts.

† Refers to scion rooting *above* wire.



FIG. 1.—Scion Rooting in Gravenstein Nurse Grafts on No. IX Rootstock. Left: normal root graft. Right: piece root inverted. Age 2 years; arrows indicate scion roots.

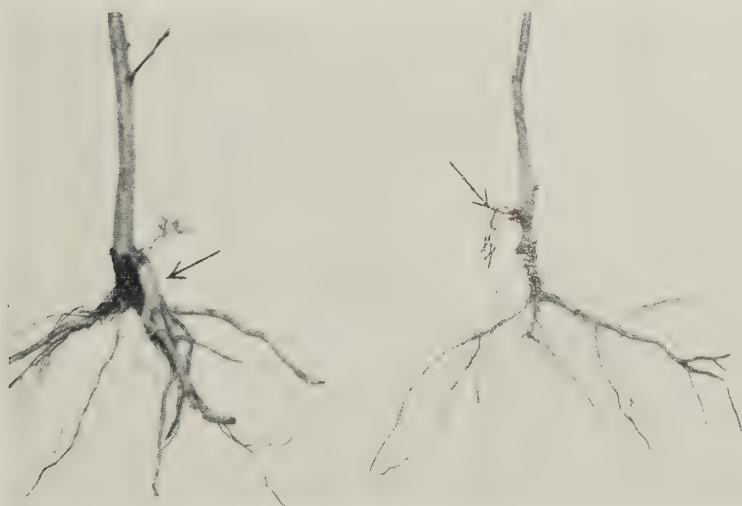


FIG. 2.—Scion Rooting in Gravenstein Nurse Grafts on Northern Spy Rootstock. Left: not wired. Right: wired. Normal root grafts; age 2 years; arrows indicate scion roots.

LOCATION OF SCION ROOTS

With the exception of wired grafts and a single graft (not wired) on Northern Spy, all scion roots originated at the extreme base of the scion, in the region of graft union (Figs. 1 and 2). This made determination of scion rooting difficult owing to close proximity, at the same level, of new lateral roots from the piece root. These lateral roots constituted a difficulty even in grafts on inverted No. IX roots, though

not present in the specimen illustrated (Fig. 1). Further, in grafts on No. IX stock—whether inverted or not inverted—the source of roots was frequently obscured by large callus galls formed at the union. Identification of scion roots was further complicated by the close resemblance of Gravenstein roots at this age to those of No. IX and Northern Spy.

With the exception of the single graft noted above, development of scion roots outside the area of graft union occurred only on wired grafts (Fig. 2). Wiring induced formation of small, bunched roots above the wire, usually immediately below a node and arising at one to three nodes.

The inverted-root graft illustrated in Fig. 1 shows stunting of the piece root below the union and absence of lateral roots, as previously observed by Kerr (1935). In other inverted-root grafts, however, lateral roots arose from the piece root in the region of graft union, i.e. at the original basal end of the piece root.

Owing to the tendency of scions to form roots only in the area of graft union, removal of the whole nurse root, before planting trees in the orchard, would be difficult to carry out without injury to or loss of scion roots. In this respect scion rooting of nurse grafts compares unfavourably with that induced by layering. The disadvantage can be overcome by wiring but scion roots formed on grafts wired in the second year were weak (Fig. 2), and a further season in the nursery would have been required for satisfactory development. The effect of wiring in the first year should be investigated.

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PREHEATING OF CREAM BEFORE VACREATOR VACUUM-PASTEURIZATION IN RELATION TO THE QUALITY OF BUTTER

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Summary

Preheating of fair to good quality cream to 150°F. or to 190°F. in tandem flash pasteurizers or to 180°F. in a plate pasteurizer before treatment in a vacreator vacuum pasteurizer did not affect the quality of the butter made from it. This applied both for the fresh butter and for the butter after four months' storage.

INTRODUCTION

The process of Vacreator vacuum-pasteurization of cream may be divided into three stages: (a) Heating of the cream to pasteurizing temperature by direct injection of steam; (b) Steam distillation under partial vacuum to remove off-flavours from the cream; (c) Cooling of the cream by further direct evaporation of portion of the water present, under higher partial vacuum. Stages (a) and (c) were later additions in the development of the Murray Vacreator vacuum-pasteurization system of cream treatment. The earlier "deodorizers" were designed to treat cream heated to pasteurization temperature in the tandem beater-type flash pasteurizers then in common use in New Zealand factories. Replacement of the flash pasteurizers by direct steam heating of the cream, as in the pasteurizer section of the Vacreator, led to dilution of the cream with condensed water. The cooler section of the Vacreator was designed to remove portion of the added water and at the same time to reduce the load on the water cooler.

While in New Zealand and Australian buttermaking practice tandem flash pasteurizers have been to a large extent replaced by Vacreators, a number of factories still use flash pasteurizers for preheating the cream before Vacreator treatment. This has one definite advantage, viz., that the degree of dilution of the cream with water is reduced. If the temperature of preheating is higher than the temperature of the cream as it leaves the cooler section there is actually some concentration of the cream in its passage through the Vacreator. The preheating has the further effect that it prolongs the period of holding of the cream at the higher temperature. While there is ample evidence (1, 2, 4) that treatment of cream in the Vacreator at 200-204°F. gives an adequate destruction of the bacteria that may be present, it is possible that the longer period of holding at the high temperature may increase the extent of destruction of enzymes. Townley and Gould (3) have shown that heating of milk and cream at high temperatures leads to formation of sulphide compounds which are mainly responsible for the cooked flavour in milk products. Some New Zealand butter factory managers have maintained that cream preheated before Vacreator treatment yields a butter of better quality than cream passed in the cold state direct into the Vacreator, but there are no published results of comparisons of butters made from creams from the same bulk supply with and without preheating.

EXPERIMENTAL

The comparisons were made in the experimental factory at the Dairy Research Institute (N.Z.). Sufficient cream was obtained for three experimental churnings. The cream was bulked in one vat, and was then divided into three portions which were treated as follows:—

Churning 1.—Cream treated in a "Baby" Vacreator to a temperature of 202-204°F.

Churning 2.—Cream heated to 150°F. by passage through two beater-type tandem flash pasteurizers in succession before delivery to the "Baby" Vacreator. The extent of steam distillation, i.e. the temperature rise in the condenser-ejector tail-water, and other conditions of Vacreator treatment were maintained as for Churning 1.

Churning 3.—Cream flash-pasteurized as for Churning 2, but to 190°F., before delivery to the "Baby" Vacreator. Vacreator treatment conditions as for Churning 1.

Comparisons were made on twelve days spread throughout the buttermaking season. The butters were graded in the fresh state at the Dairy Research Institute (N.Z.), and by the Department of Agriculture Graders at the Wellington and Auckland Grading Stores, and they were re-examined after four months' storage at 14°F. by the Department of Agriculture Graders at Wellington.

TABLE I.—COMPARISONS OF GRADINGS OF FRESH BUTTERS FROM CHURNINGS 1, 2 AND 3.

Method of Cream Treatment.	Average Grade Scores for 12 Comparisons			Average of all Gradings.
	Dairy Research Institute (N.Z.)	Wellington Graders.	Auckland Graders.	
Vacreator only	92.88	92.23	92.97	92.66
Preheating at 150°F. before Vacreator treatment	92.69	92.29	92.47	92.48
Preheating at 190°F. before Vacreator treatment	92.87	92.47	92.64	92.66

Gradings of Fresh Butters

The results given in Table I show that there was no significant difference in the quality of the freshly made butters from the three churnings.

TABLE II. GRADINGS OF 12 SAMPLES OF BUTTER AFTER STORAGE IN 56 LB. BOXES AT 140°F. FOR 4 MONTHS

Churning No.	Method of Cream Treatment.	Average Grade for 12 Churnings of Butter.
1.	Vacreator only.	91.25
2.	Preheating at 150°F. before Vacreator Treatment.	91.15
3.	Preheating at 190°F. before Vacreator Treatment.	91.42

Gradings of the Butters after Storage (see Table II)

The grade scores for the butters from cream preheated at 150°F. were again, on the average, slightly lower than for Churnings Nos. 1 and 3, but the difference cannot be regarded as of any practical significance.

Use of Plate Pasteurizer for Preheating

In factories where plate machines are used as water cream-coolers the preheating may be effected in a separate section of the machine, either by use of Vacreator condenser-ejector tail-water or by means of steam-heated hot water from a reservoir. Some comparisons were made of this closed system of preheating against preheating to 180°F. with flash pasteurizers, and against normal Vacreator treatment. There was no evidence that use of the plate pasteurizer for preheating had any disadvantage over use of flash pasteurizers, even with acid cream which required heavy addition of neutralizer to reduce the acidity to 0.08 per cent lactic acid, although the practical operation of the plate preheater with this latter type of cream was less simple than with ordinary flash pasteurizers.

DISCUSSION

The results described above refer to creams which were of fair to good quality. They do not provide any evidence as to whether claims made by some factory operators that preheating of feedy cream makes the feedy flavour more difficult to remove in the Vacreator are valid. They do show, however, that with cream of fair quality there is no disadvantage to quality through use of the flash-pasteurizer or plate preheater. The decision as to whether the cream should be preheated before delivery to the Vacreator can therefore be made on economic and practical grounds, i.e., does the reduction in volume of cream to be churned, and the consequent saving in labour costs and in butterfat losses in the buttermilk justify the extra cost involved?

ACKNOWLEDGMENTS

The authors desire to thank the Department of Agriculture Grading Staff at Auckland and Wellington for assistance in examination of the butters.

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RESISTANCE OF LINSEED AND LINEN FLAX VARIETIES TO FLAX RUST (*Melampsora lini*) IN NEW ZEALAND

PART I

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(Received for publication, 12 January 1949)

Summary

(1) Fourteen varieties of linseed and seven of linen flax were tested for resistance to flax rust (*Melampsora lini*).

(2) In the linseed group Sheyenne, Punjab, and Golden Viking were resistant, while Koto, Moose, Victory, Walsh, Minerva, Redwing, Registered Royal, Royal, Bison, Golden Indian, and Indian showed moderate to high degrees of susceptibility.

(3) Of the linen-flax group, three Russian varieties (Stakanovetz, D-83, and 806/3), Wada, Cascade, and Argentine II were resistant, while Liral Crown was susceptible.

INTRODUCTION

FLAX rust (*Melampsora lini* (Ehrenb.), Lev.) was first recorded on linen flax (*Linum usitatissimum* L.) in New Zealand by Cunningham (1927). Subsequently this disease has been present to a greater or lesser extent each season. War conditions made necessary a large increase in acreage of linseed and linen flax. At the same time the practice of sowing linseed and linen-flax crops both in the autumn and spring was introduced.

Cunningham (1944) in an unpublished report concluded that rust was the most serious disease present in linen-flax crops, and the only one of economic significance during the 1943-44 season. Last season (1947-48) a flax-rust epiphytotic was experienced.

To help relieve this position and to obtain varieties which might be more suitable than the present commercial lines, a number of new varieties have been imported in recent years. These have been introduced by the Agronomy Division, Department of Scientific and Industrial Research, and Dominion Industries Ltd. In this paper an account is given of the results obtained in Auckland from testing these and locally grown varieties of linseed and linen flax for rust resistance.

EXPERIMENTAL METHOD

Individual physiologic rust races, as such, were disregarded. Uredospore collections of flax rust (*M. lini*) were made during the late autumn (April 1948) from the chief linseed and linen-flax-growing areas of New Zealand, viz. Methven, Makikihi, Washdyke, Geraldine, Winton, and Fairlie. A collection was also made from Lincoln. All races of rust of economic importance to linen flax and linseed industries in the Dominion were probably present in these collections.

The seven collections were kept separate, and supplies of uredospores were increased by artificial inoculation of the susceptible Liral Crown. Uredospores were stored prior to use in 1 dram glass phials at 5°-7°C.

Inoculation procedure was based upon the recommendations of Waterhouse and Watson (1941), and Flor (1935). Flax plants were grown in four-inch-diameter pots under heated glasshouse conditions (19° - 24° C.). When plants were four to six inches high they were inoculated with a mixture of uredospores. The latter consisted of approximately equal amounts of uredospores from each collection, mixed thoroughly with an equal amount of bentonite as a diluent. The inoculum was distributed by evenly dusting plants with a camel-hair brush. Following inoculation, pots were immediately transferred to a humidity cabinet for 24 hours. This was kept at 16 - 17° C. and 98-100 per cent relative humidity. Subsequently the pots were moved to glasshouse benches where they remained until uredosori had developed and were ready for counting. Twelve to fifteen days after inoculation, counts were made of the total number of uredosori on three leaves* of each plant.

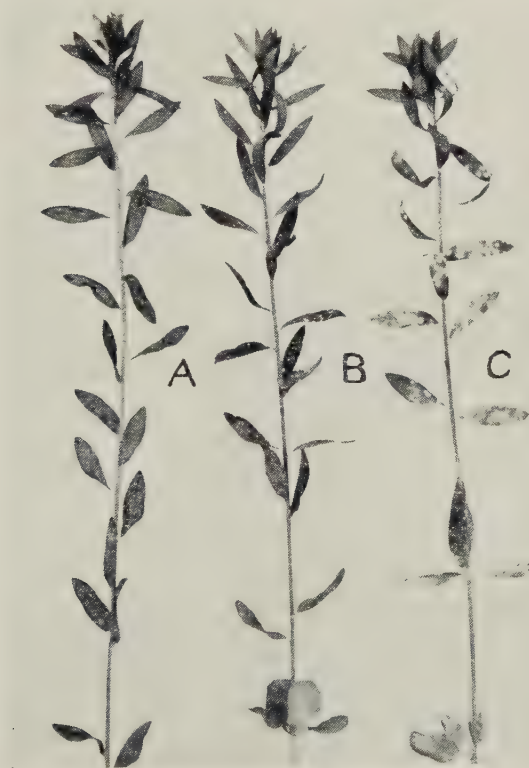


FIG. 1.—Rust-inoculated Linseed Plants.

Photo: A. I. Hughes.

- A. Highly resistant variety (Sheyenne).
- B. Resistant variety (Golden Viking).
- C. Highly susceptible variety (Royal).

*A leaf in the centre of the most heavily rusted region was selected, with a second two to three nodes above and the third an equal distance below.

Six trials at different dates, involving from 6 to 12 varieties in each, were carried out, and with the exception of two varieties each variety test was repeated on three occasions. In each trial, ten plants were grown per pot, six pot replications were made of each variety, while the pots were arranged at random in trays. Results were subjected to statistical analysis.

RESULTS

Results are set out in Table I. In Fig. 1 are shown typical inoculated plants of a highly resistant variety, Sheyenne, a resistant variety, Golden Viking, and a highly susceptible variety, Royal. These degrees of infection were used as an infection scale for classification of varieties into the broad infection groups given in the final column of Table I.

TABLE I. VARIETAL SUSCEPTIBILITY OF LINSEED AND
LINEN FLAX TO FLAX RUST (*MELAMPPOA LINEI*)

LINSEED					
Variety *	No. of Trials	Variety Grand Means of Uredosorus Counts per Pot of Ten Plants	Standard Error	Significance	General Grading
Sheyenne	3	0			Highly Resistant
Punjab (a)	3	0		x	
Punjab (b)	3	2.2	± 0.8		
Punjab (c)	3	2.9	± 0.3		Resistant
Golden Viking (a)	3	31.4	± 3.2	xx	
Golden Viking (b)	3	31.7	± 4.4		
Golden Viking (c)	3	38.3	± 5.1	xx	Susceptible
Koto (a)	3	124.9	±17.2		
Koto (b)	3	132.5	±18.5	x	
Moose	1	227.3	±36.6		
Victory (a)	3	237.8	±15.2		
Victory (b)	3	265.6	±31.0		
Walsh (a)	3	310.0	±27.9		Highly Susceptible
Walsh (b)	3	315.1	±28.8		
Minerva (X Canada)	1	421.8	±42.5		
Minerva (X U.S.A.)	1	502.8	±51.4		
Redwing	3	659.5	±33.2	x	
Regd. Royal	3	672.3	±26.8		
Royal (a)	3	684.8	±20.4		
Royal (b)	3	713.2	±26.3		
+ Bison	3	820.4	±23.1	xx	
Golden Indian	3	890.6	±56.8	xx	
Indian	3	1093.2	±40.9		
LINEN FLAX					
Stakhanovetz	3	0			Highly Resistant
D-83	3	0			
806/3	3	0			
++ Argentine II	3	0			
++ Wada	3	0			
++ Cascade	3	0		xx	
+ Liral Crown	3	366.8	± 9.3		Susceptible

* Lines within a variety are designated by small letters.

+ Commercial varieties most commonly grown in New Zealand up to 1947-48 season.

++ A few plants in each trial were highly susceptible and are not included in the above results (see text).

CONCLUSIONS

Analysis of the above results (Table I) shows that there are in New Zealand a number of varieties resistant to flax rust. These are Sheyenne, Punjab*, and Golden Viking (linseed); three Russian varieties (Stakhanovetz, D-83, 806/3), Wada, Cascade, and Argentine II (linen flax). In the last three varieties one to two per cent of the plants in each trial were heavily rusted. This was considered to be caused by stray seeds of other varieties in the seed samples supplied for testing, and therefore they were not penalized on this account.

ACKNOWLEDGMENTS

The author wishes to acknowledge assistance given by officers of the Agronomy Division, Department of Scientific and Industrial Research, by the management, Dominion Industries Ltd., and by factory managers, Linen Flax Corporation of New Zealand. Assistance with the statistical analysis of results by Mr. H. R. Thompson, Applied Mathematics Laboratory, Department of Scientific and Industrial Research, is also acknowledged.

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* In recent trials, using flax rust collected from infected Punjab, this variety showed high susceptibility.

ZINC CONTENT OF LEAVES AND FRUIT SPURS OF APPLE TREES

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(Received for publication, 25 October 1949)

Summary

Samples of apple leaves, fruit spurs and leader growth have been analysed for zinc in the case of Gravenstein, Cox's Orange, Sturmer and Jonathan varieties of apples grown in different parts of the Nelson district. There were marked differences in the zinc content of the different types of samples subjected to analysis, the leaf samples giving the lowest and the spur bark samples the highest figures. With the exception of certain peculiarities associated with the Gravenstein variety, there were no consistent differences in zinc status shown by the four varieties of apples. Apple leaves gave the most consistent results in assessing the zinc status of the trees.

INTRODUCTION

A suspected case of zinc deficiency in apple trees at Tasman in the Nelson district made it desirable to obtain data on the zinc content of apple leaves and different parts of fruit spurs and leader growth, with a view to ascertaining what samples of apple material were most suitable for zinc determinations, and to obtain standards for zinc in different varieties of apples which could be used for comparison both with overseas data and with the zinc content of apple material collected in New Zealand.

Collection of Samples

EXPERIMENTAL

Samples of leaves, fruit spurs and leader growth were collected from Gravenstein, Cox's Orange, Sturmer and Jonathan varieties of apples from orchards located at Stoke, Mahana, Tasman, Braeburn, Redwood's Valley and Waimea West. The fruit spur samples were carefully separated into fruit buds, spur bark and spur wood. The leader growth samples were similarly separated into leader bark and leader wood. All the samples were collected from the same blocks of trees, but the number of leaf samples was increased by sampling additional orchards. The samples were dried and carefully ground to avoid zinc contamination.

Zinc Estimation

Estimation of zinc in the apple samples was carried out according to the method Cholak, Hubbard and Burkey¹ using a slight modification reported in a previous paper.²

RESULTS

The results of the zinc estimations are shown separately for the different varieties in Table I.

DISCUSSION

Great differences in zinc content were found in the different types of apple material subjected to analysis. The leaf samples gave the lowest and most consistent results, the average zinc content being 31 p.p.m. with a narrow range of 25 to 33 p.p.m. for the four varieties examined. Leader wood was slightly higher in zinc content than the apple leaf samples, the average zinc content for the four varieties being 36 p.p.m. compared with 31 p.p.m. for corresponding leaf samples. The leader bark samples gave greatly increased zinc figures, the average for the four varieties being 73 p.p.m.

TABLE I. DISTRIBUTION OF ZINC (EXPRESSED IN PARTS PER MILLION ON DRY MATTER BASIS) IN FRUIT SPURS AND LEADER SHOOTS IN:

Locality	Leaves	Spur Buds	Spur Bark	Spur Wood	Leader Bark	Leader Wood
(A) GRAVENSTEIN APPLES						
Stoke	29	105	494	187	63	38
Mahana	32	55	487	94	60	24
Tasman	20	118	252	96	74	50
Braeburn	18	61	259	60	48	44
Redwood's Valley	24	69	501	176	76	37
Waimea West	26	38	100	30	63	19
Average	25	74	349	107	64	35
(B) STURMER APPLES						
Stoke	28	46	104	92	149	66
Mahana	31	115	367	83	.	.
Braeburn	26	89	142	92	98	9
Redwood's Valley	40	84	250	150	.	.
Waimea West	39	52	81	71	24	19
Average	33	77	189	97	90	31
(C) JONATHAN APPLES						
Stoke	30	33	141	67	76	41
Mahana	32	99	364	75	87	41
Braeburn	24	93	261	55	52	36
Waimea West	45	94	286	103	75	52
Average	33	80	263	75	72	42
(D) COX'S ORANGE APPLES						
Stoke	28	72	253	118	52	22
Mahana	28	108	194	97	61	38
Redwood's Valley	36	71	163	51	.	.
Average	31	84	203	89	57	30
Average for all Samples	31	79	251	92	71	35

NOTE: Zinc determinations made on leaf samples collected on a more extensive scale gave the following figures for different varieties of apples: Gravenstein (9 samples) average 25 p.p.m.; Cox's Orange (7 samples) average 29 p.p.m.; Sturmer (10 samples) average 29 p.p.m.; Jonathan (9 samples) average 32 p.p.m..

The zinc content of the fruit buds was somewhat similar to that of the leader bark samples, the average zinc content being 79 p.p.m. compared with 73 p.p.m. for leader bark.

Both spur wood and particularly spur bark gave much higher figures for zinc, average contents being 91 and 266 p.p.m. respectively.

It is a matter of interest that the zinc content of spur bark on the Gravenstein variety was considerably higher than in the other varieties, the average zinc content being 349 p.p.m. compared with 263 p.p.m. for corresponding Jonathan samples.

The determinations, apart from the high zinc content of spur bark and a tendency for somewhat lower figures for Gravenstein apple leaves, did not show any consistent differences in the zinc content of the four varieties of apples. The location of the orchards in the different parts of the Nelson district did not appear to exert any marked effect on the zinc content of the apple samples.

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EFFECT OF TEMPERATURE ON CHINESE GOOSEBERRIES IN COOL STORE

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and Industrial Research, Wellington

(Received for publication, 15 January 1949)

Summary

The investigation showed that Chinese gooseberries can be satisfactorily kept in a cool-store at 31-32°F.

Stalks should be removed to prevent punctures in adjacent berries; careful packing will prevent fruit from splitting. Losses due to *Penicillium* spp. on these damaged areas were considerable.

INTRODUCTION

At the request of a commercial cold-storage firm a small scale investigation was made on the storage of the fruit of Chinese gooseberry (*Actinidia chinensis*). This fruit is becoming quite extensively grown in the warmer parts of New Zealand and is valued by the public for its delicate flavour and its relatively high vitamin-C content.

A search of the available literature failed to show any reference to the storage conditions required for this fruit, and the trial was therefore undertaken to determine the most suitable temperature.

EXPERIMENTAL PROCEDURE

A commercial line of Chinese gooseberries, from Mr. W. Gibbs, Otumoetai, Tauranga, Bay of Plenty, was received on 26 June 1948. It was decided that two-case samples should be held at the various temperatures available, viz. 31°F., 32°F. and 38°F. A better idea of the effect of temperature would have been gained had an intermediate temperature in this range been available.

An initial examination was not made until 29 June 1948, when the fruit was weighed and counted. It was also noted that the berries were inclined to be soft, which suggested that the storage period would be short. On 29 July 1948, the fruit was removed, reweighed and examined for storage disorders. Owing to the soft nature of the fruit held at 38°F., this line was not returned to store.

The fruit held at 31°F. and 32°F. was returned to store until 12 August 1948, when it was again weighed and examined. By this time a high percentage of the berries was too soft to return to store for a further period and the experiment was terminated.

RESULTS

Results are presented in Table I.

Differences of temperature have no appreciable effect on the loss of weight by the fruit, which was small at all temperatures. No adequate explanation can be given for the gain in weight of sample A2 after four weeks' storage. It is suspected that the initial weight was incorrectly recorded, because the weighing on 29 July 1948 was rechecked when this discrepancy was noted.

Chinese gooseberries picked in June can be stored for six weeks at 31-32°F. with less than 10 per cent loss and without the fruit becoming too soft. Similar fruit would *not* hold satisfactorily at 38°F. for a month.

The two types of skin injury noted were identified by the Plant Diseases Division as *Cladosporium herbarum*, a secondary infection on bruised areas causing a black-brown discolouration, and *Glomerella cingulata*, present as dark brown areas on parts of the fruit which bore evidence of previous mechanical or insect damage.

It is thought that neither of the diseases developed to any extent in storage, but merely became more evident as the covering hairs dropped away from the fruit. Neither disease had penetrated through to the flesh.

A high percentage of the rejected fruit was either split or punctured by stalks.

Splitting was most noticeable in the outside layers of fruit, and was probably caused by irregularities in the boards of the cases, particularly when these were too full. This trouble could be largely overcome by the inclusion of a layer of paper all round the inside of the case.

Puncture of the fruit by the stalks occurred when the latter were too long. In many of the fruits inspected the stalks were up to one inch in length, and it is considered that this type of damage could be overcome by double-clipping each berry so that the stalk is removed as close to the button as possible.

Penicillium was found mostly in the punctured or split areas. From this primary source, the fungus will spread rapidly and its presence on the skin of a number of berries indicated that this spread was already taking place. If the storage period had been longer, the disease would no doubt have penetrated below the skin of the affected berries.

It is thought that had the fruit been firmer on arrival, it could have been held for a longer period, especially at the lower temperatures.

TABLE I. THE EFFECT OF TEMPERATURE ON CHINESE GOOSEBERRIES HELD IN COOL STORE FOR 6 WEEKS

Temp. °F.	1st Examination after 4 Weeks' Storage.						2nd Examination after Further 2 Weeks' Storage.						Combined Data from 1st and 2nd Examinations.		
	Sample No.	Initial Count Per Box.	Sound Firm Fruits. (%)	Sound Soft Fruits. (%)	Rejec- ted Fruits. (%)	Weight- loss during Storage. (%)	Count for Second Period of Storage.	Sound Firm Fruits. (%)	Sound Soft Fruits. (%)	Rejec- ted Fruits. (%)	Weight- loss during Second Period of Storage. (%)	Sound Firm Fruits. (%)	Sound Soft Fruits. (%)	Rejec- ted Fruits. (%)	
31	A.1.	181	90	8	2	2.0	177	63	33	5	1.0	55	39	6	
	A.2.	144	78	19	3	6.4 (Gain)	140	48	49	4	2.5				
32	B.1.	173	90	8	2	2.0	169	57	40	2	.6	49	44	8	
	B.2.	157	72	24	4	2.3	150	42	51	7	1.0				
38	C.1.	148	38	40	22	2.8						44	39	17	
	C.2.	179	49	38	13	2.1									

NOTES ON FRUIT REJECTED :

Sample No. (a) After First Examination.

No.

A.1. Too soft for further storage.

A.2. 100 soft for further storage.

B.1. 3 too soft for further storage, 1 split with primary penicillium.

B.2. 1 split, 1 punctured, 3 badly bruised, 2 with penicillium on skin only.

C.1. 1 split, 10 with primary penicillium, 22 with penicillium on skin only.

C.2. 4 split, 1 punctured, 4 with black mould, 9 with primary penicillium, 7 with penicillium on skin only.

(b) After Second Examination.

4 split, 2 split with penicillium, 2 with primary penicillium.

Stem punctured.

1 punctured, 2 with primary penicillium, 1 with penicillium on stem only.

10 punctured, 1 with primary penicillium.

DAIRY COW EFFICIENCY

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(Received for publication, August 1949)

Summary

1. The diminishing increment curve for level of production against feed intake published by Jensen and associates has been transposed to fit dairy cows with body weights and butterfat tests typical for New Zealand dairy stock. From this the gross total digestible nutrient (T.D.N.) requirement per pound of butterfat per annum has been calculated for varying levels of production.

2. As total butterfat production per annum is raised, the increase in net quantity of digestible nutrients required to produce an extra pound of butterfat is such that the overall benefit gained from the greater spread of non-productive maintenance overhead is progressively reduced and finally eliminated. Above that level every extra pound of butterfat becomes increasingly expensive in terms of total nutrients required.

3. It is suggested that for an average dairy cow of 800 lb live weight and milk of 5.0 per cent butterfat test, this level of maximum efficiency is probably about 270 lb butterfat per annum.

4. The probable effects of variations in butterfat test and body weight on level of maximum efficiency and gross efficiency (T.D.N. per pound butterfat) are assessed.

5. Further data published by Jensen and associates provide a basis for illustrating the influence of dairy merit on efficiency of production.

INTRODUCTION

The view that the butterfat production of a very large proportion of New Zealand dairy cows is considerably restricted by poor feeding is fairly widely held and has often been quite forcibly expressed. In discussing the improvements in production which can be obtained by management, as distinct from breeding, major emphasis has been given to the need to increase production per cow. It has been pointed out that the higher producing animal is more economical to run, for the initial non-productive requirement of food for maintenance can be spread over a greater number of pounds of butterfat, thus reducing the overall feed requirement per pound of butterfat. From this has come the statement or implication that the higher production per cow to be expected from any improvement in feeding would be nearly pure profit, since with the maintenance requirement already met, every extra pound of nutrients will be worth a given quantity of extra milk.

When the very obvious weaknesses in the average standard of feeding of New Zealand dairy cows are noted, and the numerous examples of farmers who by applying the tenets of good feeding are obtaining better than average production per cow are considered, there is justification for emphasising the need for improvement in production per cow by better farm management and better feeding.

However, when the results which are actually being obtained by dairy farmers *as a whole* are examined, there are a number of points which deserve further consideration.

(1) For New Zealand conditions the best overall index of farm productivity is "pounds of butterfat produced per acre". In both the farm surveys made about fifteen to twenty years ago by Fawcett¹ and the present series of surveys by Hamilton and Mitchell², it is found that high production of butterfat per acre is much more closely associated with high rates of stocking, i.e., carrying capacity, than with high production per cow. From the results of the most recent survey, viz. that of Waipa County³, it can be estimated that high carrying capacity is at least three times as important as high production per cow for obtaining high production per acre.

(2) Since the change-over to the Jersey and other specialized dairy breeds was completed farmers have made little, if any, increase in production per cow, despite the continued emphasis given to its importance. Though there are undoubtedly many factors responsible, this would at least suggest that over all there is rather limited scope for easily obtaining large improvements in average production per cow.

(3) As within any one herd all cows have access to the same feed supply, the question arises as to whether feed supply is the factor dominating average levels of production when it is common to find some cows producing well over 300 lb of butterfat per annum while others do not even reach the 200 lb mark. With most of these lower producing cows in a herd, it is probable that factors other than feed-supply play a major part in limiting production.

The view which regards better farm management and feeding primarily as an instrument for improving production per cow may need to be modified. For New Zealand conditions high production per cow can only be regarded as a means to an end. That end is high overall production per farm, i.e. high production of butterfat per acre. The three points mentioned above indicate that under actual farm conditions, *with present average cows*, there are a number of other factors beside better feeding of the individual animal which are of considerable importance in achieving high production per acre.

A large number of these other factors may be lumped under the heading "efficiency of feed utilization". This covers the whole sequence from the point when grass or other food is in a fit state to be eaten, up to the extraction of milk from the cow.

It may be subdivided into

(a) The proportion of the total digestible nutrients grown, which are actually eaten by the cow. It is difficult to make a distinction between measures to produce the maximum quantity of grass and ones aiming for high efficiency of its utilization.

(b) The efficiency with which the nutrients eaten are converted to butterfat. The discussion which follows considers this point only.

In a comprehensive series of feeding trials Jensen *et al.*⁴ have examined the relationship between level of feeding and milk production. Using the efficiency relationships between animals of various live weights which are postulated by Brody⁵, the author has recalculated these results to fit New Zealand conditions.

In their series of trials, Jensen *et al.*⁴ examined the level of milk production obtained by feeding dairy cows at various rationing levels. They report that "the response to heavier feeding increases at a progressively slower rate as the level of feeding is increased and is properly represented by a curve instead of a straight line. The output for added feed is more than twice as much at the lowest level as at the highest level".

To these data they fitted the exponential curve given by the Spillman diminishing-returns equation⁶. It was found for these data that the equation could be reduced to—

$$Y = A (1-R^x)^*$$

when feed consumption is expressed in terms of the *production ration alone*. The constants for the curve they fitted to the combined data for 392 cows from nine experimental stations (Fig. 1) were kindly calculated by Mr. P. J. Armstrong, Applied Mathematics Laboratory.

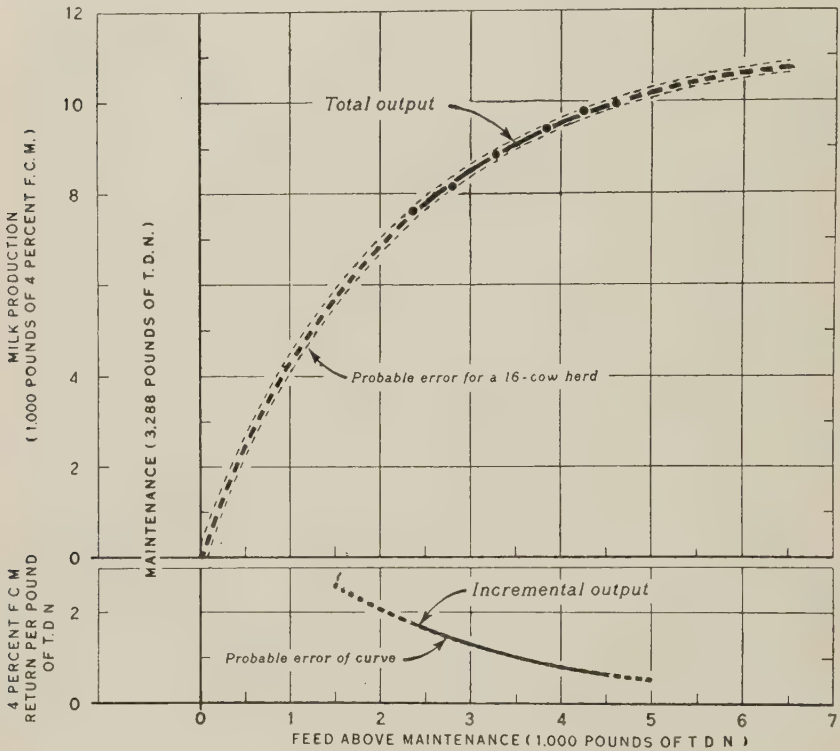


FIG. 1.—Feed input and milk output of cows fed at six different levels of feeding at nine stations (being copied from Fig. 13 of U.S. Department of Agriculture Technical Bulletin 815).

$$* x = -5000 \times \log \left\{ 1 - \frac{Y}{1134} \right\}$$

x = Feed Consumption expressed as lb of Total digestible nutrients (T.D.N.).

Y = Milk Output expressed as lb of four per cent fat corrected milk (F.C.M.).

A = Range between maximum and minimum production.

R determines the path of the curve and is a measure of the amount of milk that would be forthcoming from an additional 1000 lb of T.D.N. compared with the production that resulted from the previous 1000 lb.

These values were calculated from measurements from the published curve and are, therefore, subject to a small margin of error.

TABLE I. ASSESSMENT OF GROSS NUTRIENT REQUIREMENTS PER POUND OF BUTTERFAT FOR A COW OF 800 LB LIVE WEIGHT PRODUCING MILK WITH A 5.0 PER CENT BUTTERFAT TEST

The equation for the curve published by Jensen et al.⁴ was calculated to be -

$$X = -5000 \times \log \left(1 - \frac{Y}{1134} \right)$$
 for cows of 1,144 lb live weight.⁵

Production from cows of equivalent dairy merit varies as 0.73 power of body weight
 On this basis the above equation was recalculated to fit an 800 lb cow of equivalent dairy merit. It becomes -

$$X = -3850 \times \log \left(1 - \frac{Y}{8740} \right) \quad \text{where } X = \text{feed consumption (T.D.N.)}$$

$$Y = \text{milk production (F.C.M.)}$$

From this equation, and converting to 4 per cent fat corrected milk, the following results were obtained:

Production per Cow		Equiv. 4% milk (lb)	T.D.N. for milk Production (lb)	Net T.D.N. per lb of Butterfat (lb)	Maintenance T.D.N. (lb)	Gross T.D.N. Requirements (lb)	Gross T.D.N. per lb of Butterfat (lb)
Butterfat (lb)	Milk (lb)						
180	3600	4140	1074	5.97	2533	3607	20.03
200	4000	4600	1251	6.26	2533	3784	18.92
220	4400	5060	1448	6.58	2533	3981	18.09
240	4800	5520	1671	6.96	2533	4204	17.51
260	5200	5980	1925	7.40	2533	4458	17.14
280	5600	6440	2233	7.98	2533	4766	17.02
300	6000	6900	2603	8.68	2533	5136	17.12
320	6400	7360	3088	9.65	2533	5621	17.56
340	6800	7820	3769	11.08	2533	6302	18.53

Brody is rather sceptical whether this is the only equation that would satisfactorily fit the data but considers that "the theory and therefore the fitting of the equation of the principle of diminishing returns is, however, reasonable". Jensen states that several equations had been fitted to the observations but none gave appreciably better fit.

From these comments it would appear that though the diminishing-returns curve fitted by Jensen may not be exactly correct, it is sufficiently close to the true position to provide a reasonable basis for this essentially preliminary examination of the levels of efficiency to be expected from New Zealand dairy stock.

Assessment for New Zealand Conditions

The cows used in Jensen's experiments averaged 1,144 lb live weight with an average maintenance requirement of 3,228 lb of total digestible nutrients. Results were expressed in terms of 4 per cent fat corrected milk (F.C.M.).

The equation for the curve and also the maintenance requirements have been recalculated for animals with live weights and butterfat tests more typical of those met in New Zealand herds. From these have been calculated for various levels of production the *gross requirements of digestible nutrients (T.D.N.) per pound of butterfat produced*. The bases on which the data were recalculated were as follows:—

(a) That the average maintenance requirements of a dairy cow vary as 0.73 power of the body weight⁵.

(b) That the dairy merit or "lactational drive" of the average New Zealand dairy cow is the same as that of the 392 cows used by the U.S. Experiment Station in their feeding trials. The exact interpretation placed on the term "dairy merit" and the validity of this assumption are more fully discussed later.

(c) That if other conditions are equal, milk energy production, i.e. total milk production expressed as calories of energy, varies as 0.73 power of body weight⁵. For the basic calculations it has been necessary to think in terms of calories of energy in order to get a direct comparison between feed eaten and milk produced, and between milks of different butterfat contents.

It will be noted that if points (a) and (b) hold, this value is automatically correct. Gaines⁷ postulates that milk energy production varies directly as body weight. If Brody's 0.73 power relationship for maintenance and live weight is accepted, Gaines' postulate would imply that smaller cows are of lower dairy merit, a conclusion one would hesitate to accept.

(d) Four per cent fat corrected milk used by Jensen has been converted to its equivalent for milks of 3.5 per cent, 4.5 per cent, 5.0 per cent, 5.5 per cent and 6.0 per cent butterfat test, using the conversion factors listed by Brody⁵.

To show clearly the procedure used, the various steps are set out in full for an 800-lb cow giving milk with a 5.0 test (Table I). *From this table it will be seen that as total butterfat production per annum is raised the increase in net quantity of digestible nutrients required to produce an extra pound of butterfat is such that the overall benefit gained from the greater spread of the non-productive maintenance overhead is progressively reduced and finally eliminated. Above this point every extra pound of butterfat becomes increasingly expensive in terms of total nutrients required.*

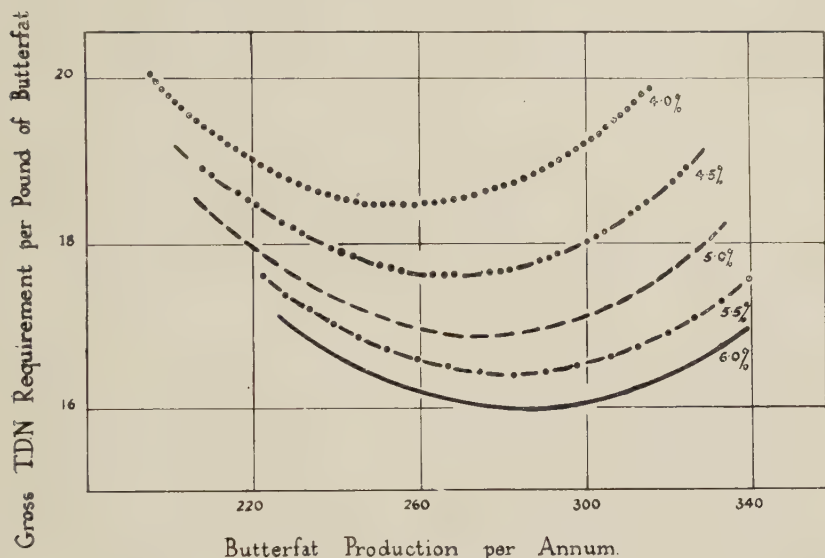


FIG. 2.—Curves of efficiency of cows of 800-lb live weight and equal dairy-merit producing milk of butterfat test indicated.

The gross T.D.N. requirements per pound of butterfat per cow of 800 lb live weight with 5.0 test milk are 18.09 lb if producing 220 lb of butterfat, 17.02 lb. for 280 lb of butterfat, and 18.53 lb for 340 lb.

This technique can now be taken a step further to examine the influence of butterfat test and body weight both alone and in combination.

Butterfat Test

The curves of efficiency for cows of 800 lb live weight and equal dairy merit, producing milk of 4.0 per cent, 4.5 per cent, 5.0 per cent, 5.5 per cent, and 6.0 per cent butterfat test are shown in Fig. 2.* Their maximum efficiency in terms of gross T.D.N. per pound of butterfat and the level of butterfat production at which this is attained is set out in Table II.** Not only does milk of higher butterfat test require less T.D.N. per pound of fat, but also the level of annual butterfat produc-

TABLE II. INFLUENCE OF BUTTERFAT TEST ON EFFICIENCY OF PRODUCTION OF AN 800-LB DAIRY COW

Butterfat Test of Milk. (%)	Level of Maximum Efficient Production of Butterfat per annum. (lb)	Maximum Efficiency. T.D.N. per lb of Butterfat. (lb)
4.0	258	18.5
4.5	273	17.7
5.0	284	17.0
5.5	293	16.4
6.0	301	16.0

* The data from which Figs. 2, 3, 4 and 6 have been drawn are held by the editor.

** In Tables II, III, IV and VI the values quoted have been read from graphed curves. This may give an error of up to 1 per cent.

tion at which the cow reaches maximum efficiency is raised. Thus an average 800 lb cow producing milk of 4.5 test reaches maximum efficiency at 273 lb of butterfat with 17.7 lb of T.D.N. per pound of butterfat. A cow of the same size giving 5.5 per cent test milk reaches maximum efficiency at 293 lb using 16.4 lb T.D.N. per pound of butterfat.

However, it will be noted that the law of diminishing returns is apparently also operating here. The higher the test the smaller the gain in efficiency from a rise of an extra half per cent.

Body Weight

The curves of efficiency for animals of equal dairy merit and the same test milk (4 per cent) but with body weights of 800, 900 and 1,144 lb are shown in Fig. 3. The bigger the animal, the more butterfat it needs to produce before reaching maximum efficiency (Table III). The T.D.N. requirement per pound of butterfat at the optimum level of production is of course the same, viz. 18.5 lb.

TABLE III. INFLUENCE OF VARIATION IN BODY WEIGHT ON EFFICIENCY OF PRODUCTION FROM COWS PRODUCING MILK OF 4 PER CENT TEST

Live Weight of Cow. (lb)	Level of Maximum Efficient Production of Butterfat per annum. (lb)	Maximum Efficiency. T.D.N. per lb of Butterfat. (lb)
800	258	18.5
900	285	
1144	335	

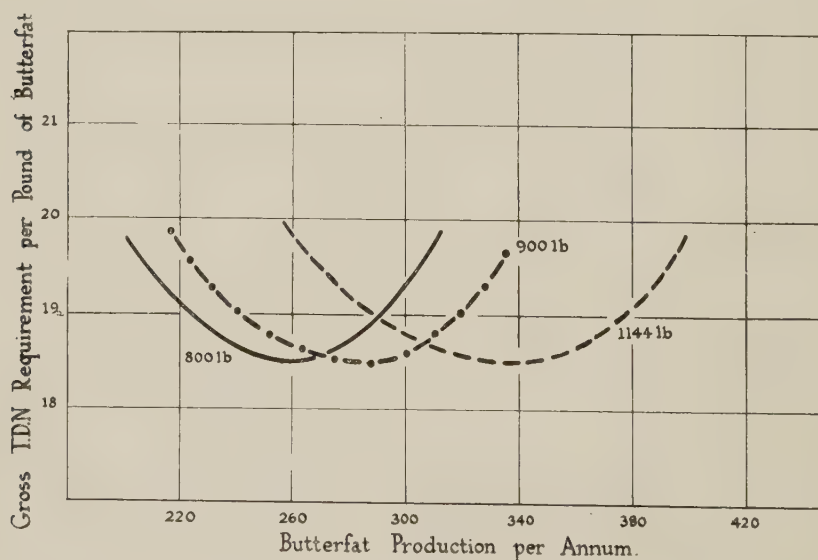


FIG. 3.—Curves of efficiency of cows of different body-weights but of equal dairy merit producing milk of four per cent butterfat test.

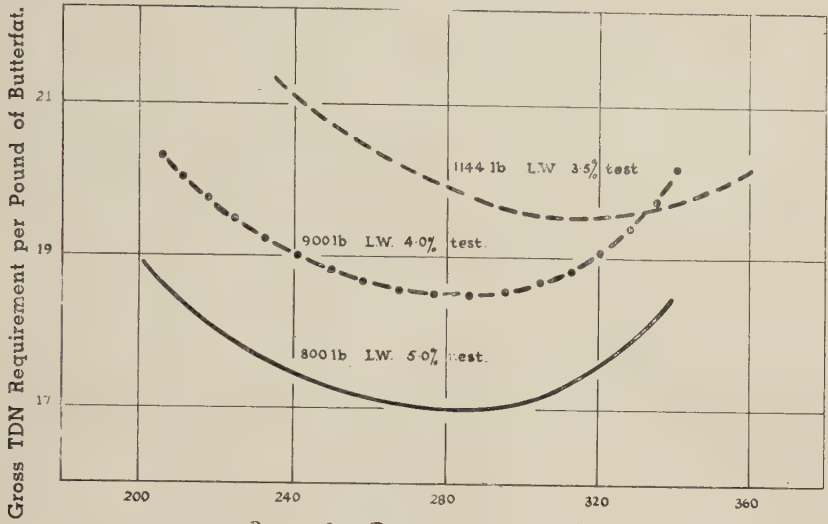


FIG. 4.—Curves of efficiency of cows of varying body weights producing milk of varying butterfat test.

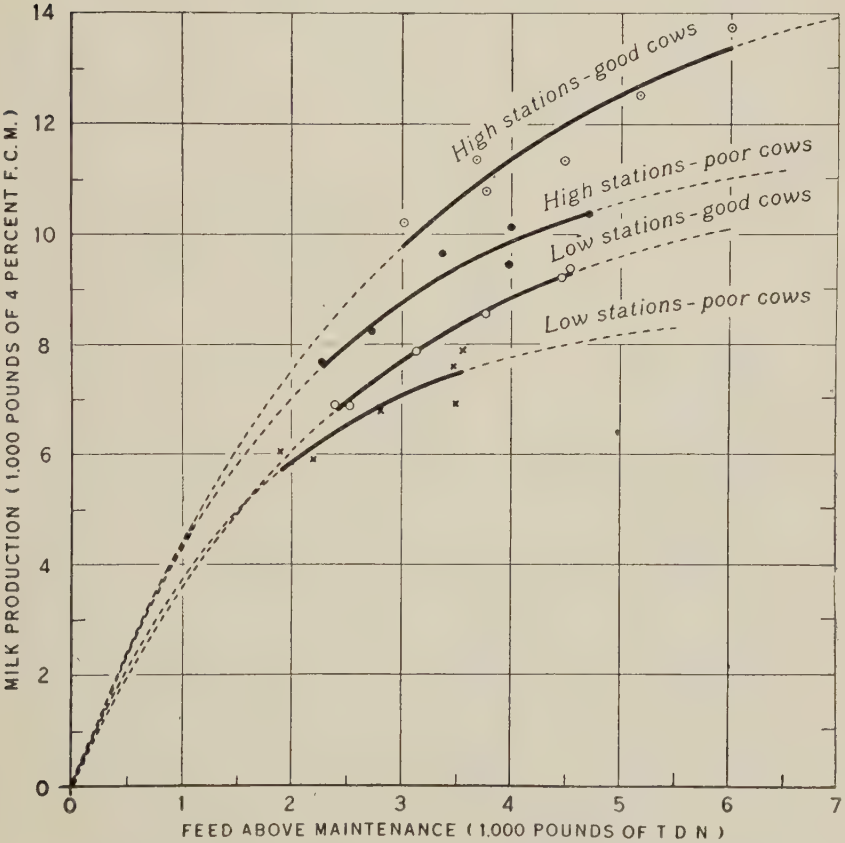


FIG. 5.—Input-output curves of cows of different inherent productivity at nine experiment stations (being copied from Fig. 14 of U.S. Department of Agriculture Technical Bulletin 815).

TABLE IV. INFLUENCE OF VARIATIONS IN BUTTERFAT TEST AND LIVE WEIGHT ON EFFICIENCY OF PRODUCTION

Live Weight (lb)	Butterfat Test (%)	Level of Maximum Efficient Production of Butterfat. (lb)	Maximum Efficiency. T.D.N. per lb of Butterfat (lb)
800	5.0	284	17.0
900	4.0	285	18.5
1144	3.5	320	19.5

Animals of Varying Body Weight and Butterfat Test

The effects of varying both body weight and butterfat test are somewhat more complex and only three examples are shown in Fig. 4.

The 900-lb cow with a 4.0 per cent test reaches maximum efficiency at the same level of butterfat production as the 5.0 per cent test 800-lb cow but requires an extra 1.5 lb of T.D.N. per lb of butterfat. The 1,144-lb cow with 3.5 per cent test requires a further 1 lb T.D.N. per lb of butterfat and does not reach maximum efficiency till producing 320 lb per annum. This is 35 lb above the level for the two smaller cows.

Dairy Merit

It is of immediate practical interest to assess the level of production at which dairy stock can be expected to reach maximum efficiency. To a very large extent this is determined by their dairy merit.

The previous discussion has been based on the assumption that average New Zealand dairy cows are of the same dairy merit as the 392 cows at the U.S. Experiment Station used in Jensen's experiments (between 25 per cent and 26 per cent). The soundness of this assumption is of considerable moment and before proceeding further it is necessary to define more precisely the concept "dairy merit" and, in the light of this definition, to examine the factors affecting it.

Brody⁵ defines dairy merit as the "biological efficiency of milk production as measured by the percentage of consumed total digestible nutrient (T.D.N.) energy which is converted into milk energy". (4 per cent fat corrected milk—F.C.M.)*

It will be seen from Table I that the calculated dairy merit of an animal will vary according to her level of feeding. Therefore, we need to add to the above definition the rider "when the cows are fed at the same rationing level, i.e. to a level where for cows of similar weight, equal percentage increase in production ration produces equal percentage increase in milk yield".

It would appear that the following are among the factors which may influence the above assumption of equivalent dairy merit:—

(I) Factors suggesting higher dairy merit among New Zealand Stock

(a) Among dairy cows there is a tendency for smaller animals to have higher dairy merit than larger animals⁵. This is probably due to selection being based, over the years, more on total production per animal

* It may be represented by the equation:

$$\text{Dairy Merit} = \frac{\text{Milk Energy Production}}{\frac{\text{T.D.N. Energy Consumption}}{340 \text{ cal} \times \text{lb of F.C.M. milk produced}}}$$

$$= \frac{\text{Milk Energy Production}}{1814 \text{ cal} \times \text{lb of T.D.N. consumed}}$$

than on true dairy merit, i.e. unless smaller animals were of higher dairy merit than larger animals, they tended to be eliminated from a herd. This trend is more marked within any one breed than between breeds. However, it would appear from Brody's data that American Jerseys may have about 2 per cent greater dairy merit than American Holsteins.⁵

(b) Average production of New Zealand pedigree Jersey cows appears to be only a few per cent above that of grade cows.

(c) The live weight of a mature New Zealand Jersey cow is probably lower than that of the Jersey cows used in assessing American results. This may imply a gain in dairy merit.

II. *Factors suggesting lower Dairy Merit among New Zealand Stock*

(a) The dairy efficiency of young stock is considerably lower than for mature animals, other things being equal. Instead of a 1 per cent increase in live weight during growth being accompanied by a 0.73 per cent approximate increase in milk production as would occur if the $W(0.73)$ relationship held, there is a milk increase of well over 1 per cent. The rate of increase suggested by Brody⁵ appears to be greater than that suggested by Ward's maturity equivalent conversion factors⁸. Both, however, are well above the 1 per cent level.

Jensen's trials included no animals which had not completed at least one lactation, whereas New Zealand dairy herds contain about 20 per cent of first-lactation heifers.

(b) All cows used in Jensen's trials were from U.S. Experiment Station herds. It is known that several of the Stations co-operating have very good herds and over-all, the dairy merit of the cows used was probably better than that in U.S. commercial dairy herds using the same breeds, viz. Holstein and Brown Swiss.

Balancing the points outlined above, it would appear that we can expect the dairy merit of the average mature cow in New Zealand herds to be about equal to that of Jensen's cows. The high proportion of young stock may make the overall figure lower. Should this be the case, the level of production for maximum efficient production (Tables I and II) would also be lowered.

The extra energy used for grazing and walking may influence the position, for it would raise the cow's maintenance requirements. Considering Hancock and Wallace's data⁹ in relation to average dairy farm conditions, it may be estimated that the additional energy required by a Jersey cow would add about 10 per cent to annual maintenance requirements. In so far as this represents extra non-productive feed intake, it could be expected to lower efficiency. On the other hand, the extra maintenance overhead would raise the level of production at which maximum efficiency is reached. The net effect on level of maximum efficiency of production is probably less than 5 per cent and for the present is not allowed for in the graphs.

Comparisons Between Good and Poor Cows

There is undoubtedly a wide range of variation between individual cows, and between individual herds. This is well illustrated by the data for some hundreds of cows published by Brody⁵. This author states that the dairy merit of good dairy animals is about 25 per cent, for superior animals, 33 per cent, and that the upper limit for any animal is probably 50 per cent, i.e. not over half the consumed T.D.N. energy can be converted to milk energy. The highest level recorded by Brody was 48 per cent.

Jensen *et al.*⁴ also publish diminishing increment curves for groups of high and low producing cows in good and poor herds. (Fig. 5).^{*} The fit of the data to these curves is not particularly good, but remembering the quite good fit obtained for the curve applied to the combined data for all cows, it would appear reasonable to fit similar curves to these data. Even though they might have been slightly modified if each group had included considerably larger numbers of cows, these curves do give a good illustration of the differences between good and poor cows, and between high and low producing herds.

TABLE V. INFLUENCE OF DAIRY MERIT ON EFFICIENCY OF PRODUCTION FROM A COW OF 800-LB LIVE WEIGHT WITH 5.0 PER CENT TEST MILK

Dairy Merit Group	Level of Maximum Efficient Production of Butterfat per annum (lb)	Maximum Efficiency. T.D.N. per lb of Butterfat. (lb)
Poor cows Low producing herds	220	20.3
Good " " " "	270	18.6
Poor " High " " "	300	16.5
Good " " " " "	360	14.6

Dairy merit may be considered in terms of the slope and upper limit of the increment curve. The increment curve for a cow of high dairy merit would be initially steeper or stay steep for a longer period and would reach higher levels of production before it became level or nearly so.

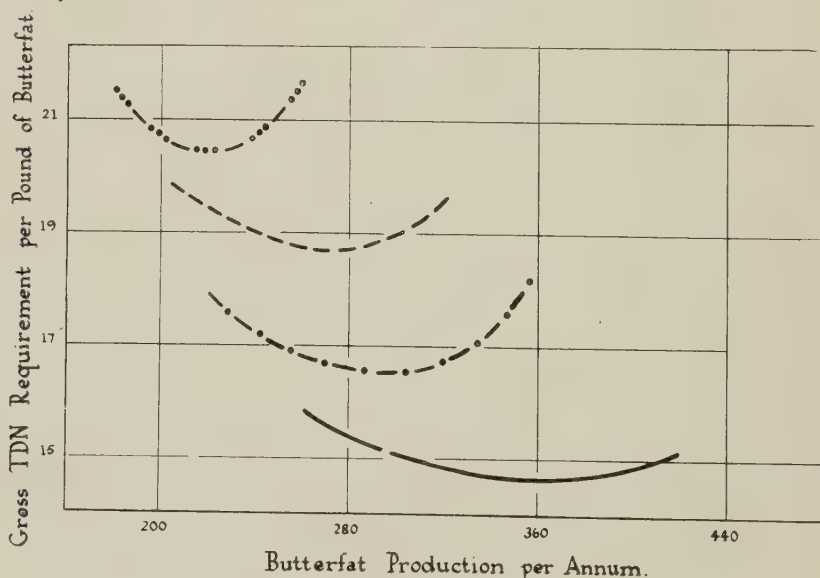


FIG. 6.—Curves showing gains in efficiency and levels of production expected from breeding better stock.

^{*} The differences between the herds would be due partly to environmental and partly to genetic differences. Whatever the relative influence of any one of the factors concerned, the net effect gives considerable difference in effective dairy merit.

When these curves are transposed to fit cows of 800 lb live weight with a 5.0 per cent butterfat test and the results expressed in terms of gross efficiency per pound of butterfat, the position is as shown in Fig. 6 and Table V. Not only do cows of higher dairy merit require much less feed per pound of butterfat produced, but they also reach maximum efficiency at a much higher level of production.

It would also suggest that it is much easier to raise the production of a cow of high dairy merit by better feeding than that of a cow of low dairy merit. While the most efficient group is within half a pound of optimum T.D.N. utilization from 300 to 420 lb butterfat, the least efficient is within this distance of optimum feed utilization only between 195 and 245 lb, and above that range its efficiency falls away much more sharply. These figures are quoted merely to illustrate the point and cannot be given any precise significance for such is not warranted by the accuracy of the original curve fitting.

The above situation fits well with a number of observations which have been made within the dairy industry and has interesting implications.

(a) Hancock and Wallace⁹ in their classical observations at Ruakura found a very poor relationship between the level of production and the average grazing time of a cow, even though average grazing time appeared to give quite a good indication of the dry matter intake. Within a group of 10 cows run together, there was 100 per cent difference in production, but only 10 per cent difference in grazing time.

(b) Even though the same feed supply (pasture, hay, etc.) is available to all cows within a herd, there are frequently cows producing well above or below herd average. Similarly, the ability of a good herd sire to considerably increase production even though management and feeding remain constant, is well known.

These increment curves would imply that such situations are to be fully expected among cows of varying dairy merit. Even when cows are fed uniform quantities of nutrient (T.D.N.) above maintenance requirement, those of higher dairy merit will produce more milk and give higher production per acre.

(c) From cows of higher dairy merit, such as the progeny of merit bulls, not only can production be altered at given levels of feeding, but there will also be greater productive response from additional feed. A cow of high dairy merit will produce more milk from an extra pound of nutrients (T.D.N.) than will a cow of low dairy merit.

DISCUSSION

While it is not possible to state definitely that the slope or shape of the Jensen curve⁴ for the relationship between level of feeding and butterfat production would be the same for a representative sample of New Zealand dairy cows, there is little doubt that the principle of diminishing returns from increased feeding does operate for butterfat production, and that the type of curve which fits the New Zealand dairy herd is probably not radically different from that given by the cows used by Jensen.

The very real gains in efficiency and level of production to be expected from breeding better stock are illustrated by Fig. 6 and Table V. However, a study of the position by a committee of the Herd Improvement Council¹⁰ and the results obtained to date from progeny testing and artificial insemination¹¹ have shown that the use of breeding and selection to effect marked improvement in the national dairy herd is essentially a long term process. Therefore, when giving immediate consideration to the problem of improving dairy production it must be accepted that for some years to come, farmers will be using stock of not very much higher dairy merit than at present.

On balancing the various points it would appear that the stock in New Zealand dairy herds are of slightly lower dairy merit than those used in Jensen's feeding trials. Therefore, the average New Zealand Jersey cow would probably reach maximum efficiency of butterfat production about the level of 270 lb per annum using for that, 17.0 lb of T.D.N. per pound of butterfat. This is not very much above the levels already being attained in an average-to-good season in the better dairying districts. Should the requirements for grazing be greatly in excess of the 10 per cent over maintenance requirements postulated earlier, this figure of 270 lb of fat could be materially reduced, e.g. if grazing requirements proved to be 50 per cent above maintenance requirements the figure would be approximately 340 lb.

When standard of feeding is considered, there are at least two major points which may substantially affect the position:

(1) The level of feeding of most New Zealand dairy cows is very uneven, whereas Jensen's cows were fed on an even standard of rationing throughout the year.

(2) Wallace¹² has clearly shown that for pregnant ewes the plane of nutrition prior to lambing affects the development of the udder tissue and subsequent milking capacity. The same can be expected to hold for dairy cows, and for New Zealand conditions, as it has already been shown that the level of pre-calving feeding does influence subsequent production.¹³

Without overlooking these and related aspects, and without in any way suggesting that improvement in feeding does not everywhere require attention, it may still fairly be suggested from the evidence presented that in the better dairying districts the scope for further improvement in efficiency *per pound of butterfat* is probably not very great.

This assessment remains very much of a conjecture and final conclusions must await the assembling of input-output data not only for superior cows under superior management but also for average cows under average management conditions.

From this approach it would also follow that for dairy stock on a low plane of nutrition, improvement in feeding will produce big improvements in production, but for average animals on a fairly high plane of nutrition, equivalent improvement in feeding will produce much smaller increases. Improvement of dairy merit, i.e. breeding, becomes of much more importance.

There is the concept that on the great majority of dairy farms an increase in carrying capacity would almost automatically improve the utilization of available grass, and, if accompanied by reasonable pasture management practices, would also tend to increase total pasture production. This view is well supported by the results of dairy farm surveys^{1, 2, 3, 14.}

Therefore, even if the above suggestions are only approximately correct, it may be stated that attempts to improve gross production per farm, by management, will show greatest results if the farmer sets out to improve carrying capacity. Once herds of average merit have reached the 260-270-lb level, the improvement in efficiency of butterfat production per pound of feed eaten will become very small indeed.

ACKNOWLEDGMENTS

The development of the ideas presented in this paper owe much to discussions the author had with many people. He wishes to thank Dr. W. M. Hamilton, Professor I. L. Campbell and staff and officers at the Animal Research Station, Ruakura, for their valuable comments and constructive criticism. He also wishes to thank the United States Department of Agriculture for permission to copy Figs. 13 and 14 of Technical Bulletin 815.

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NOTE ON THE REACTION OF ACTIVATED GLYCEROL
DICHLOROHYDRIN WITH CAROTENE AND ITS
OXIDATION PRODUCTS

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INTRODUCTION

Activated glycerol dichlorohydrin (GDH) as first introduced by Sobel and Werbin^{1,2} is finding increasing use as a colorimetric reagent for the estimation of vitamin A, its chief advantages over the more usual Carr-Price reagent being that the colour produced is stable for from 2 to 10 minutes while the reagent is non-corrosive and is not hygroscopic. It has been used for the estimation of vitamin A in a wide range of materials and has been found to give results in good agreement with those obtained spectroscopically and with the Carr-Price reagent³.

An investigation carried out recently in this Department necessitated the estimation of vitamin A in the presence of partially oxidized carotene. It is well recognized that the Carr-Price reagent is not specific for vitamin A and that it reacts with related substances including carotene and its breakdown products. Johnson and Baumann⁴ have shown that the intensity of the blue colour produced with carotene solutions depends on the state of oxidation of the sample. No information was, however, available regarding the reaction of GDH with substances related to vitamin A and carotene⁵, and it was thought that it might possess some advantages over the Carr-Price reagent in this direction. Its reaction with carotene at various stages of oxidative decomposition was therefore investigated. For comparison, apparent vitamin A was also estimated by means of the Carr-Price reagent and spectroscopically by measuring the absorption at 325 μ .

EXPERIMENTAL

A solution of carotene in peroxide-free ethyl ether was exposed to diffuse sunlight at room temperature. At intervals two samples were withdrawn simultaneously and evaporated separately to dryness in a stream of nitrogen at room temperature. The residue from one sample was dissolved in petroleum ether and the absorption measured at 325 μ and 450 μ . The other residue was taken up in chloroform. One millilitre of this solution was treated with 4 ml of GDH—activated shortly before use by the addition of 2 per cent hydrochloric acid as recommended by Penketh⁶, and the colour intensity measured 3 minutes after mixing at 555 μ . A further 1 ml of the chloroform solution was treated with 8 ml of a solution of antimony trichloride in chloroform (25 per cent w/v). All extinction measurements except those involving the Carr-Price reagent were made using a Beckman Spectrophotometer calibrated against standard solutions of pure vitamin A alcohol. In the case of the Carr-Price reagent a colorimeter of the Evelyn type was used. This was fitted with a red filter and calibrated using solutions of vitamin A alcohol in chloroform. The instrument was adjusted to read 100 per

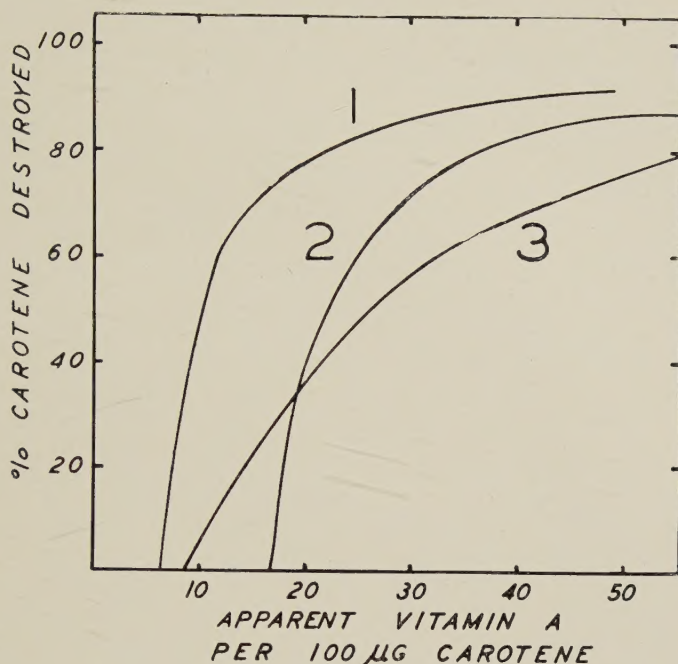


FIG. 1.—Apparent vitamin A at different stages of decomposition of a solution of carotene as estimated by the Carr-Price reagent (Curve 1), Glycerol dichlorohydrin (Curve 2) and spectroscopically (Curve 3).

cent transmission on a control cell containing chloroform. A cell containing the 1 ml of chloroform solution together with 2 drops of acetic anhydride was moved into the light path and the reagent added from a rapid delivery pipette. Percentage transmission was measured 3 seconds after addition of the reagent.

The percentage carotene destroyed was estimated from the decrease in the readings at 450μ and apparent vitamin A at different stages of decomposition was calculated from the results obtained by these three methods of assay. These results are expressed in $\mu\text{g}/100\mu\text{g}$ carotene in Fig. 1. It is clear that GDH reacts with decomposition products in the same way as the Carr-Price reagent over which it does not appear to possess any advantages in this respect, the removal of decomposition products by chromatography being necessary in both cases. Despite the increase in apparent vitamin A, an estimation of true vitamin A based on a measurement of the absorption at 325μ is still possible with reasonable accuracy even in the presence of heavily oxidised carotene by the application of a three point correction procedure (7) since wavelengths may be selected above and below 325μ such that the points on the absorption curve of the oxidised carotene corresponding to these three wavelengths are co-linear.

The values obtained for apparent vitamin A per $100\mu\text{g}$ undecomposed carotene (90 per cent β and 10 per cent α) were $6.4\mu\text{g}$ for the Carr-Price reagent, $16.8\mu\text{g}$ for GDH, and $8.52\mu\text{g}$ for the spectroscopic method.

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